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(54) Title: UROCORTIN PEPTIDES

(57) Abstract

Urocortin (Ucn) is a native mammalian peptide generally related to Urotensin I and Corticotropin Releasing Factor (CRF). Human Ucn has the formula: Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 (SEQ ID NO:15). Rat-derived Ucn is identical but for 2 substitutions, Asp^2 for Asn^2 and Pro^4 for Ser^4. Ucn or analogs thereof or pharmaceutically acceptable salts can be administered to humans and other mammals to achieve substantial elevation of ACTH, β-endorphin, β-lipotropin, other products of the pro-opiomelanocortin gene and corticosterone. They can also be used to lower blood pressure over an extended period of time, as stimulants to elevate mood and to improve memory and learning performance, as well as diagnostically. Shortened fragments may be administered to release endogenous CRF and/or Ucn in the brain and peripherally. Ucn antagonists can be used to block the action of Ucn and/or CRF, as can antibodies to Ucn. Labelled Ucn agonists and antagonists can be used in drug screening assays along with CRF receptors; they may also be used diagnostically along with Ucn antibodies.

UROCORTIN PEPTIDES

This invention was made with Government support under
Grant Number DK-26741 awarded by the National Institutes of
Health. The Government has certain rights in this
invention.

5   This application claims priority from Provisional
Application Serial No. 60/002223, filed August 11, 1995 and
from Serial No. 08/490,314, filed June 13, 1995, which is
being converted to a provisional application.

This invention is directed to peptide hormones, to
10 methods for treatment of mammals, including humans, using
such peptides, to antibodies which bind such peptides, to
methods for diagnosis and drug screening using such
peptides and/or antibodies, and to nucleic acid encoding
such peptides. More specifically, the invention relates to
15 a native peptide having certain pharmacological properties
in common with urotensin and with CRF, which is termed
urocortin (Ucn), to analogs and fragments thereof (broadly
termed Ucn-like peptides), to pharmaceutical compositions
containing such Ucn peptides and to methods of treatment of
20 mammals, method of diagnosis and methods of screening using
such Ucn peptides and antibodies thereto.

BACKGROUND OF THE INVENTION

Experimental and clinical observations have supported
25 the concept that the hypothalamus plays a key role in the
regulation of adenohypophysial corticotropic cells
secretory functions. Although over 40 years ago,
Guillemin, Rosenberg and Saffran and Schally independently
demonstrated the presence of factors in hypothalamus which
30 would increase the rate of ACTH secretion by the pituitary
gland incubated in vitro or maintained in an organ culture,
a physiologic corticotropin releasing factor (CRF) was not
characterized until ovine CRF (oCRF) was characterized in
1981. It was disclosed in U.S. Patent No. 4,415,558, as
35 having the amino acid sequence (SEQ ID NO:1):

H-Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-
Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Thr-Lys-Ala-Asp-Gln-Leu-
Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala-
NH_2.

5   Although originally isolated and characterized on the
basis of its role in this hypothalamopituitary-adrenal
(HPA) axis, CRF has been found to be distributed broadly
throughout the central nervous system as well as in
extraneural tissues, such as the adrenal glands, placenta
10 and testes, where it may also act as a paracrine regulator
or a neurotransmitter. Moreover, the likely involvement of
CRF in affective disorders, such as anxiety, depression,
alcoholism and anorexia nervosa, and in modulating
reproduction and immune responses suggests that changes in
15 CRF expression may have important physiological and
pathophysiological consequences. For example,
perturbations in the regulatory loops comprising the HPA
axis often produce chronically elevated levels of
circulating glucocorticoids; such patients display the
20 physical hallmarks of Cushing's syndrome, including truncal
obesity, muscle-wasting, and reduced fertility.
        In addition to its role in mediating activation of the
hypothalamic-pituitary-adrenal, CRF has also been shown to
modulate autonomic and behavioral changes, some of which
25 occur during the stress response. Many of these behavioral
changes have been shown to occur independently of HPA
activation in that they are not duplicated by dexamethasone
treatment and are insensitive to hypophysectomy. In
addition, direct infusion of CRF into the CNS mimics
30 autonomic and behavioral responses to a variety of
stressors. Because peripheral administration of CRF or a
CRF antagonist fails to affect certain of these changes, it
appears that CRF exhibits a direct brain action with
respect to such functions, which include appetite
35 suppression, increased arousal and learning ability.
However, CRF antagonists given peripherally attenuate
stress-mediated increases in ACTH secretion, and when

delivered into the cerebral ventricles can mitigate stress-
induced changes in autonomic activity and behavior.

As a result of the extensive anatomical distribution
and multiple biological actions of CRF, this regulatory
5 peptide is believed to be involved in the regulation of
numerous biological processes. CRF has also been
implicated in the regulation of inflammatory responses.
Although it has been observed that CRF plays a pro-
inflammatory role in certain animal models, CRF appears to
10 suppress inflammation in others by reducing injury-induced
increases in vascular permeability.

In about 1981, a 40-residue amidated peptide generally
similar to CRF was isolated from the skin of the South
American frog *Phyllomedusa sauvagei*; it is referred to as
15 sauvagine. It was characterized by Erspamer et al. and was
described in <u>Regulatory Peptides</u>, Vol. 2 (1981), pp. 1-13.
Sauvagine has the amino acid sequence (SEQ ID NO:2):
pGlu-Gly-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Ser-Leu-Glu-Leu-Leu-Ar
g-Lys-Met-Ile-Glu-Ile-Glu-Lys-Gln-Glu-Lys-Glu-Lys-Gln-
20 Gln-Ala-Ala-Asn-Asn-Arg-Leu-Leu-Leu-Asp-Thr-Ile-NH_2. When
given intravenously(iv), sauvagine and oCRF have been
reported to cause vasodilation of the mesenteric arteries
so as to lower blood pressure in mammals and also in
stimulating the secretion of ACTH and β-endorphin.
25 However, when administered intracerebroventricularly(icv),
there is an elevation of heart rate and mean arterial blood
pressure, which are secondary to activation of the
sympathetic nervous system.

Rat CRF(rCRF) was isolated, purified and characterized
30 in about 1982-1983 as a hentetracontapeptide having the
amino acid sequence (SEQ ID NO:3):
H-Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-
Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-
Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-
35 NH_2. The formula of human CRF was subsequently determined
to be the same as that of rCRF. The compound is often

referred to as r/hCRF and is covered in U.S. Patent No.
4,489,163.

At about the same time, two homologous polypeptides
were isolated from the urophyses of different species of
5 fish.   These isolated peptides were generally homologous to
CRF, i.e. about 54% homology, and were termed Urotensin I
(UI).   Catostomus commersoni (white sucker or suckerfish) UI
is a polypeptide having the amino acid sequence (SEQ ID
NO:4):

10 H-Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-
Leu-Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg-Ile-Glu-Asn-Glu-
Arg-Glu-Gln-Ala-Gly-Leu-Asn-Arg-Lys-Tyr-Leu-Asp-Glu-Val-
NH$_2$; it is sometimes referred to as suckerfish(sf)
urotensin or sfUI.   Its purification and characterization

15 are described in an article by Lederis et al., Science Vol.
218, No. 4568, 162-164 (Oct. 8, 1982).   The homolog, carp
urotensin, was obtained from Cyprinus carpio and has the
amino acid sequence (SEQ ID NO:5):
H-Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-

20 Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg-Asn-Glu-Asn-Gln-Arg-
Glu-Gln-Ala-Gly-Leu-Asn-Arg-Lys-Tyr-Leu-Asp-Glu-Val-NH$_2$.
Another urotensin homolog having the following amino acid
sequence (SEQ ID NO:6):
H-Ser-Glu-Glu-Pro-Pro-Met-Ser-Ile-Asp-Leu-Thr-Phe-His-

25 Met-Leu-Arg-Asn-Met-Ile-His-Arg-Ala-Lys-Met-Glu-Gly-Glu-
Arg-Glu-Gln-Ala-Leu-Ile-Asn-Arg-Asn-Leu-Leu-Asp-Glu-Val-
NH$_2$ was later isolated from the urophyses of
Hippoglossoides elassodon or Flathead (Maggy) Sole; it is
sometimes referred to as Maggy urotensin.   Synthetic UIs

30 have been found to also stimulate ACTH and β-endorphin
activities in vitro and in vivo and to have many of the
same general biological activities of CRFs and sauvagine.

Since the discovery of the original discoveries of
CRFs in mammals and urotensins in fish, CRFs have now been

35 shown to exist in other animal species.   For example, fish
CRF was found to be a 41-residue peptide having high

homology to r/hCRF; it has the amino acid sequence (SEQ ID
NO:7):   H-Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-
Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu
-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Met-Met-Glu-Il
5 e-Phe-NH$_2$.   Synthetic fish CRF (fCRF) stimulates ACTH and
β-endorphin activities *in vitro* and *in vivo* and has similar
biological activities to mammalian CRFs.   Because of the
high homology between fCRF and r/hCRF, it is thought that
other mammalian hormones may exist which would be the
10 counterparts of urotensin and/or sauvagine.

SUMMARY OF THE INVENTION

Another peptide 40 residues in length has now been
discovered, which is related to urotensin and CRF; it is
15 arbitrarily referred to as urocortin (Ucn).   It has less
than 50% homology with rat/human CRF.   Although it has the
same length as sauvagine, it shares less than 40% homology
with sauvagine.   It has 62.5% homology with the closest
urotensin sequence, i.e. carp urotensin.   Thus Ucn has less
20 than about 80% homology with any other previously known
native peptide.   Rat Ucn has the following amino acid
sequence (SEQ ID NO:8):   Asp-Asp-Pro-Pro-Leu-Ser-Ile-Asp-
Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-
Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-
25 Phe-Asp-Ser-Val-NH$_2$.   Human Ucn has the following amino
acid sequence (residues 83-122 of SEQ ID NO:15):   Asp-Asn-
Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-
Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-
Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$.   Thus, hUcn is
30 the same as rUcn except for Asn^2 and Ser^4.   Ucn has
biological properties which are considered to generally
resemble those of known CRFs, urotensins and sauvagine but
is more biopotent in a number of respects.

The present invention provides Ucn-like peptides,
35 including human and rat Ucn and Analogs thereof, which have
substantially all the properties of known CRFs.   These Ucn-
like peptides not only are potent hypotensive agents, but

they have additional pharmacological and physiological
properties over and beyond those of heretofore known CRFs.
More specifically, agonists are provided for the
stimulation of the known CRF receptors (referred to as CRF-
5 Rs), i.e., CRF-R1 and CRF-R2 and their splice varients, as
well as the putative novel receptor for Ucn.

Ucn competitive antagonists are also provided which
bind the CRF-Rs and the putative Ucn receptor with high
affinity but do not significantly stimulate or activate
10 such receptors. Such antagonists are broadly created by
deleting a sequence of from 7 to 10 residues beginning at
the N-terminus from the amino acid sequence of Ucn or from
an analog sequence that is substantially the same.
Preferably 9 or 10 residues are deleted, and most
15 preferably 9 are deleted. It may be preferred that the
shortened N-terminus be acylated with a group having 7 or
less carbon atoms, e.g. $[Ac-Thr^{10}]$-Ucn(10-40), and the
inclusion of one, two or three other residues at the N-
terminus, e.g., Pro^{10}, does not markedly affect biopotency.
20 Other substitutions may be effectively made as described
hereinafter. Particularly, $D-Phe^{11}$ or $D-Tyr^{11}$ may be
present at the N-terminus and/or a lactam bond created
between residues 29 and 32. These antagonists can be
administered to achieve at least the same physiological
25 effects as the known CRF antagonists, and such more
effective methods of treatment are thus provided.

Fragments of Ucn and Ucn Analogs that are useful to
block CRF-binding protein (CRF-BP) are further provided
which are effective to elevate levels of endogenous
30 peptides normally cleared by the binding protein. More
specifically, these Ucn-like peptides and blocking
fragments bind to human CRF-binding protein with high
affinity and effectively compete with human CRF and human
Ucn (hUcn) in the formation of complexes with hCRF-BP; in
35 this manner, they increase the effective *in vivo*
concentration of endogenous hCRF and/or hUcn, as well as
the effective concentration of any CRF agonist or CRF

-6-

antagonist that may be optionally administered along
therewith for the purpose of achieving a particular
therapeutic purpose. As a result of blocking the effect of
CRF-BP, these fragments effectively increase the
5 concentration of endogenous CRF in those regions of the
body where CRF-BP is normally present.

The invention also provides pharmaceutical
compositions which include such Ucn-like peptides, or
nontoxic salts thereof, dispersed in a pharmaceutically
10 acceptable liquid or solid carrier. The administration of
such peptides or pharmaceutically acceptable salts thereof
to mammals, particularly humans, in accordance with the
invention may be carried out for regulation of the
secretion of ACTH, β-endorphin, β-lipotropin, other
15 products of the pro-opiomelanocortin (POMC) gene and
corticosterone and/or for lowering blood pressure or
increasing coronary flow and/or decreasing swelling and
inflammation and/or for affecting learning, mood, behavior,
appetite, gastrointestinal and intestinal functions and
20 autonomic nervous system activities.

The invention also provides antibodies which recognize
Ucn, and assays for practically employing Ucn and analogs
and/or such antibodies for the evaluation of the status of
pituitary, cardiovascular, reproductive, hepatic, immune,
25 gastrointestinal or central nervous system functions. For
example, such antibodies can be used diagnostically to
monitor the level of therapeutically administered Ucn, to
facilitate the maintenance of therapeutically effective
amounts thereof, as well as for the diagnosis of potential
30 physiological disorders that result from abnormal levels of
Ucn. Antibodies of the invention may be therapeutically
administered to neutralize endogenous Ucn; alternatively
DNA encoding such antibodies might be employed in gene
therapy. Anti-Ucn antibodies can also be used to purify
35 CRF-R protein as well as to therapeutically counteract the
biological action of Ucn *in vivo.*

The invention also provides competitive binding assays
which are particularly useful for screening candidates for
new drugs, e.g. to identify new Ucn-like peptides or other
compounds having even greater or more selective binding
5 affinity for CRF receptors and/or for CRF-BP than Ucn,
which candidates would therefore be potentially useful as
drugs. Such screening assays may be used to screen for
potential agonists of Ucn, and other assays employing a
labelled Ucn antagonist with high affinity may be used to
10 screen for more potent antagonists of Ucn. In addition,
there is provided a method for screening for particularly
effective peptides or other compounds which will block the
ability of CRF-BP to bind to CRF and Ucn and therefore
increase the concentration of CRF and/or Ucn in locations
15 where hCRF-BP is present.

The present invention further provides nucleic acid
hybridization probes in the form of isolated nucleic acid
encoding native rat Ucn and isolated nucleic acid encoding
native human Ucn, which are useful for detecting other Ucn-
20 encoding nucleic acids in biological samples or in
libraries of other species in order to identify additional
native Ucn or Ucn-like peptides. Such nucleotide sequences
also can be used as coding sequences for the recombinant
expression of complete Ucn-like peptides or desired
25 biologically active fragments thereof. Fragments of Ucn-
encoding nucleic acid can also be employed as primers for
PCR amplification of Ucn-encoding DNA. In addition,
sequences derived from sequences encoding Ucn or analogs
thereof can also be used in gene therapy applications to
30 target the expression of vectors carrying useful genes to
specific cell types, and antisense polynucleotides that
hybridize with Ucn mRNA may also be used to reduce Ucn
levels to counteract certain conditions, e.g. Ucn-secreting
tumors. More specifically, the present invention further
35 provides isolated nucleic acids encoding Ucn as well as Ucn
analogs containing L-isomers of the 20 natural amino acids.
Such nucleic acids comprise:

-8-

(a) nucleic acids that encode the amino acid sequence
of rat Ucn set forth in SEQ ID NO:8 and that encode the
amino acid sequence of human Ucn set forth in SEQ ID NO:15;

(b) nucleic acids which hybridize to the nucleic acids
5 of (a) wherein said hybridizing nucleic acids encode
biologically active Ucn-like peptides; or

(c) nucleic acids which encode fragments of Ucn or
analogs thereof which are CRF-antagonists or CRF-BP
blockers.

10

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific
terms used herein have the same meaning as is commonly
understood by one of skill in the art to which this
15 invention belongs. The nomenclature used to define the
peptides is that specified by Schroder & Lubke, "The
Peptides", Academic Press (1965) wherein, in accordance
with conventional representation, the amino group appears
to the left and the carboxyl group to the right. The
20 standard 3-letter abbreviations to identify the alpha-amino
acid residues, and where the amino acid residue has
isomeric forms, it is the L-form of the amino acid that is
represented unless otherwise expressly indicated, e.g. Ser
= L-serine. The nucleotides, which occur in the various
25 nucleic acids, are designated with the standard
single-letter designations used routinely in the art.

The term "homology" is used in its usual and well
known sense of indicating correspondence between members in
a sequence, e.g. either on an amino acid (AA) level or at
30 the nucleotide level. For purposes of this application,
the term homologous refers to at least about 70%
correspondence, the term substantially homologous refers to
a correspondence of at least about 80%, and the term highly
homologous refers to a correspondence of at least about 90%
35 or preferably about 95% or higher. The term "homolog" is
generally considered to include analogous proteins,
peptides and DNA sequences from other mammalian species

wherein insignificant changes have evolved but the homolog
still performs the same biological function in
substantially the same way.

Protein, polypeptide and peptide are used to designate
5 linear sequences of amino acid residues connected one to
the other by peptide bonds between the alpha-amino and
alpha-carboxy groups of adjacent residues. The term
polypeptide may be used interchangeably with peptide and
with the term protein; unless otherwise limited, protein is
10 generally used to describe a sequence of about 75 or more
residues.

The term "analog" includes any polypeptide having an
amino acid residue sequence generally identical to a
sequence specifically shown herein, e.g. rUcn or hUcn,
15 wherein one or more residues has been replaced (with at
least about 80% and preferably at least about 90% of the
residues being the same) and wherein the analog displays
the ability to biologically mimic the parent molecule as
described herein in some particular function. Preferably,
20 most if not all of such substitutions are replacements of a
residue with a functionally similar residue, i.e.
conservative substitutions. Examples of such conservative
substitutions include: the substitution of one non-polar
(hydrophobic) residue, such as isoleucine, valine, alanine,
25 glycine, leucine or methionine for another non-polar
residue; the substitution of one polar (hydrophilic)
residue for another polar residue, such as arginine for
lysine, glutamine for asparagine, threonine for serine; the
substitution of one basic residue such as lysine, arginine
30 or histidine for another basic residue; and the
substitution of one acidic residue, i.e. aspartic acid or
glutamic acid, for the other. The phrase "conservative
substitution" is also intended to include the use of a
chemically derivatized residue in place of a
35 non-derivatized residue provided that the resultant
polypeptide displays the requisite biological activity,
e.g. binding activity. For purposes of this application,

two peptides are considered to be substantially the same
when they only differ from each other by conservative
substitutions. Examples of preferred conservative
substitutions are set forth in Table 1.

5

TABLE 1

Original Residue	Preferred Conservative Substitutions	Most Preferred Substitution
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Lys; Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Nle	Leu
Leu (L)	Nle; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Leu; Val; Ile; Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Nle	Leu

"Chemical derivative" refers to a subject polypeptide
having one or more residues chemically derivatized by
reaction of a functional side group. Such derivatized
polypeptides include, for example, those in which free
5 amino groups have been derivatized to form amine
hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy
groups, t-butyloxycarbonyl groups, chloroacetyl groups or
formyl groups. Free carboxyl groups may be derivatized to
form salts, methyl and ethyl esters or other types of
10 esters or hydrazides. Free hydroxyl groups may be
derivatized to from O-acyl or O-alkyl derivatives. The
imidazole nitrogen of histidine may be derivatized to form
N-im-benzylhistidine. Chemical derivatives also include
those peptides which contain one or more naturally
15 occurring amino acid derivatives of the twenty standard
amino acids. For example: 4-hydroxyproline may be
substituted for proline; 5-hydroxylysine may be substituted
for lysine; 3-methylhistidine may be substituted for
histidine; homoserine may be substituted for serine; and
20 ornithine may be substituted for lysine. Peptides embraced
by the present invention also include peptides having one
or more residue additions and/or deletions relative to the
specific peptide whose sequence is shown herein, so long as
the modified peptide maintains the requisite biological
25 activity.

As used herein, the terms "pharmaceutically
acceptable", "physiologically tolerable" and grammatical
variations thereof, as they refer to compositions,
carriers, diluents and reagents, are used interchangeably
30 and represent that the materials are capable of
administration to a mammal without the production of
undesirable physiological effects such as nausea,
dizziness, gastric upset and the like.

The term "biologically active fragment" as used herein
35 refers to (a) a fragment of a peptide of the invention
which has been truncated with respect to either the N- or
C-termini, or both; or (b) a fragment of nucleic acid

-12-

corresponding to a coding region for rUcn or a highly homologous native peptide of another mammalian species which has been truncated at the 5' or 3' end, or both, and is useful in antisense applications. The peptide fragment

5 shown performs substantially the same function or a directly related biological function as does the parent.

The phrase "modulating the transactivation of CRF receptors" as used herein refers to administering a therapeutically effective amount of a physiologically

10 tolerable composition containing a Ucn-like peptide to thereby modulate CRF actions in mammals by means of direct or induced antagonistic(competitive) association with CRF receptors (CRF-Rs).

CRF-R is used to refer to a family of receptor protein

15 subtypes which participate in the G-protein-coupled response of cells to CRF and Ucn-like ligands. CRF-Rs are coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters. The G-proteins associate with the receptor proteins at the

20 intracellular face of the plasma membrane. An agonist binding to a CRF-R catalyzes the exchanges of GTP for GDP on the α-subunit (G-protein "activation"), resulting in its dissociation and stimulation of one (or more) of the various signal-transducing enzymes and channels. G-protein

25 preferentially stimulates particular effectors, and the specificity of signal transduction may be determined, therefore, by the specificity of G-protein/receptor interaction. CRF-R proteins mediate signal transduction through the modulation of adenylate cyclase and perhaps

30 through PI turnover. For example, when CRF or Ucn binds to and activates the CRF-R, adenylate cyclase causes an elevation in the level of intracellular cAMP. An effective bioassay for evaluating whether a test compound is capable of elevating intracellular cAMP is carried out by culturing

35 cells containing cDNA which expresses CRF receptor proteins in the presence of a potential agonist or antagonist whose ability to modulate signal transduction activity of CRF

-13-

receptor protein is sought to be determined. Such
transformed cells are monitored for either an increase or
decrease in the level of intracellular cAMP which provides
a determination of the effectiveness of the potential
5 agonist or antagonist. Methods for measuring intracellular
levels of cAMP, or measuring cyclase activity, are well
known in the art.

A human CRF receptor was the first to be reported, and
it was cloned from a human Cushing pituitary tumor as
10 described in Chen R., et al, *P.N.A.S.*, *90*, 8967-8971
(October 1993). It is referred to as hCRF-R1 or hCRF-RA
and has 415 amino acids; a splice variant thereof includes
an insert of 29-amino acids. A rat CRF receptor was
isolated, approximately contemporaneously, by hybridization
15 from a rat brain cDNA library. It is referred to as rCRF-
R1; it has the 415 amino acid sequence which is set forth
hereinafter as SEQ ID NO:10. It was disclosed in Perrin,
M., et al., *Endocrinology*, *133*, 3058-3061 (1993). It was
found to be 97% identical at the amino acid level to the
20 human CRF-R1, differing by only 12 amino acids. The
receptor has since been reported to be widely distributed
throughout the brain and the pituitary and to be likely
present in the adrenals and spleen.

A second subclass of CRF receptors has more recently
25 been found, and such receptors are arbitrarily referred to
herein as CRF-R2 but are sometimes referred to as CRF-RB.
One such receptor, having the amino acid sequence set forth
hereinafter as SEQ ID NO:11, was obtained by the cloning
and characterization of a cDNA from a mouse heart cDNA
30 library. It is 431 amino acid residues in length, and the
details of the receptor are set forth in Perrin, M., et
al., *P.N.A.S*, *92*, 2969-2973 (March 1995). It is
hereinafter referred to as CRF-R2β, but has been referred
to as CRF-RB$_L$.
35   Another, slightly shorter receptor of this second
subclass was independently obtained from a rat hypothalamus
cDNA library. It is referred to herein as CRF-R2α and has

the 411-amino-acid-residue-sequence set forth hereinafter
as SEQ ID NO:12. The details of its cloning are set forth
in Lovenberg, T., et al., *P.N.A.S.*, *92*, 836-840 (January
1995), wherein a second spliced variant was also identified
5 via PCR as being a putative protein of 431 amino acids that
would be the rat homolog of mCRF-R2β identified above. The
431 amino acid sequence is set forth hereinafter as SEQ ID
NO:13 and can be seen to be homologous with mCRF-R2β. The
distribution of Ucn throughout the rat brain is consistent
10 with its being the endogenous ligand for CRF-R2, as is the
fact that it exhibits a much higher binding affinity, than
does CRF, for the receptor, particularly the R2s which is
believed to be the main CRF-R in the brain.

   Ucn-like peptides, including rUcn, hUcn and analogs
15 thereof, can be easily synthesized as described in Example
I hereinafter and then individually tested for binding
affinity. Binding affinity refers to the strength of
interaction between ligand and receptor. To demonstrate
binding affinity for a CRF receptor, the peptides of the
20 invention are easily evaluated using a tracer ligand of
known affinity, such as ^{125}I-radiolabeled oCRF, in binding
assay experiments which are well known in this art. The
results of such assays indicate the affinity at which each
Ucn-like ligand binds to a CRF receptor, expressed in terms
25 of K_i, an inhibitory binding affinity constant relative to
such a known standard. K_i (inhibitory binding affinity
constant) is determined using a "standard" or "tracer"
radioactive ligand and thus measures the displacement of
the tracer from the receptor or binding protein; it is most
30 properly expressed with reference to such tracer. So long
as these assays are carefully performed under specific
conditions with relatively low concentrations of receptor
or the like, the calculated K_i will be substantially the
same as its dissociation constant K_D. It is particularly
35 efficient to test for K_i because only a single tracer need
be labelled, e.g. radioiodinated. Dissociation constant K_D
is representative of the concentration of ligand necessary

to occupy one-half (50%) of the binding sites of a receptor
or the like. A given ligand having a high binding affinity
for a CRF receptor will require the presence of very little
ligand to bind at least 50% of the available binding sites
5 so that the K_D value for that ligand and receptor will be a
small number. On the other hand, a given ligand having a
low binding affinity for a particular CRF receptor will
require the presence of a relatively high level of the
ligand to bind 50% of the sites, so that the K_D value for
10 that ligand and receptor will be a large number.

      With respect to a particular receptor protein, a Ucn-
like peptide having a K_D of about 10 nM or less means that
a concentration of the ligand (i.e., the Ucn-like peptide)
of no greater than about 10 nM will be required to occupy
15 at least 50% of the active binding sites of the receptor
protein. Such values may be fairly determined from the
results obtained using a radioiodinated standard and no
more than approximately 0.8 nM of the receptor
(approximately 10-20 pmol receptor/mg membrane protein).
20 Preferred Ucn-like peptides have a binding affinity (K_D)
such that a ligand concentration of about 10 nanomolar or
less is required in order to occupy (or bind to) at least
50% of the receptor binding sites, and particularly
preferred Ucn-like peptides have a binding affinity of 1 nM
25 or less. Generally, a dissociation constant of about 5
nanomolar or lower is considered to be an indication of
fairly strong affinity, and a K_D of about 1 nanomolar or
less is an indication of very strong affinity. For
example, rUcn binds CRF-R1 with very strong affinity,
30 having a K_D = about 0.18 nanomolar and binds CRF-R2β with
similar strong affinity. It is also considered to be
particularly advantageous to provide Ucn-like peptides
which have a substantially higher affinity for CRF-R2,
compared to CRF-R1, and which will thus be selective in
35 their biological effect. Because CRF-R2 receptors are
distributed widely throughout the body, Ucn will have a
substantially greater effect than CRF in modulating many

-16-

peripheral actions, and because the native peptide or
fragments thereof should not be immunogenic, it should be a
very good drug physiologically.

These binding assays employing CRF receptors are
5 straightforward to perform and can be readily carried out
with initially identified or synthesized peptides to
determine whether such peptides are effective agonists of
CRF, or alternatively to determine whether other shortened
candidates are effective antagonists of CRF. Such binding
10 assays can be carried out in a variety of ways as well
known to one of skill in the art. A detailed example of
such an assay is set forth in Perrin, M., et al.,
Endocrinology, 118, 1171-1179 (1986). Competitive binding
assays employing Ucn are particularly contemplated to
15 evaluate whether candidate peptides are effective agonists
with respect to each of the receptors previously described,
i.e. CRF-R1, CRF-R2β and CRF-R2α as well as assays with Ucn
antagonists to determine whether candidates are effective
antagonists. In such assays, Ucn can be appropriately
20 labeled with a substance that is readily detected, such as
a radioactive isotope, e.g. ^{125}I, or an enzyme or some other
suitable tag. For example, suitably labelled agonists,
such as ^{125}I-Tyr^{0}-Ucn, or suitably labelled antagonists,
such as ^{125}I-(cyclo 29-32)[D-Tyr^{11}, Glu^{29}, Lys^{32}]-Ucn(11-40),
25 are particularly useful tracers for use in such receptor
assays. Such receptor assays can be used as screens for
potential drugs which interact with CRF and/or CRF
receptors.

Very generally, the invention provides Ucn-like
30 peptides, including Ucn and analogs of Ucn, having an amino
acid sequence which is substantially the same as the
following amino acid sequence based upon SEQ ID NO:8 and
upon SEQ ID NO:15 (Formula I): Y-R$_1$-Pro-R$_4$-Leu-Ser-Ile-Asp-
Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-
35 Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-
Phe-Asp-Ser-Val-NH$_2$, wherein Y is an acyl group having 7 or
less carbon atoms, preferably acetyl, or hydrogen; R$_1$ is

--Asp-Asp or Asp-Asn or Asp or Asn or desR₁; and R₄ is Pro or
Ser; as well as nontoxic salts thereof. When the N-
terminus is shortened by the deletion of 2 residues, it is
preferably acylated, e.g. acetylated. These peptides have

5 pharmacological properties somewhat similar to those of
oCRF or r/hCRF and additional properties as described
hereinafter. As indicated hereinbefore, analogs of the
above having at least about 80% homology with the amino
acid sequence of either hUcn or rUcn are preferred for the

10 Ucn-like peptides of the invention, although peptides
having at least 66% homology with either hUcn or rUcn,
wherein all or all but one of the substitutions are
conservative substitutions, are considered to be
biologically active and to have advantages over known CRF

15 peptides. Particularly preferred are analogs which are
substantially the same as either hUcn or rUcn (as defined
hereinbefore) and which have D-isomer amino acid
substitutions and/or cyclizing bonds between the side
chains of specific residues in the sequence which are known

20 to increase ligand binding affinity for CRF receptors.

In addition to the foregoing general group of Ucn-like
peptides, two additional groups are disclosed hereinafter
based upon related syntheses and testing carried out in
this general area with regard to ligands which bind to CRF-

25 R.

The following group of analogs of Ucn does not merely
include one or more conservative substitutions. Instead,
bioactive Ucn analogs are found to be defined according to
the following amino acid sequence: Y-Asp-R_2-Pro-R_4-Leu-

30 Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-R_{19}-
Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-R_{29}-Ala-Glu-R_{32}-Asn-Arg-
Ile-R_{36}-Phe-R_{38}-Ser-Val-NH_2, wherein Y is an acyl group
having 7 or less carbon atoms or hydrogen; R_2 is Asp or
Asn; R_4 is Pro or Ser; R_{19} is Glu or Ala; R_{29} is Arg, Glu,

35 Lys or Orn; R_{32} is Gln, Lys, Orn or Glu; R_{36} is Ile, C$^{\alpha}$MeIle
or C$^{\alpha}$MeLeu; R_{38} is Asp or Ala; provided that when R_{29} is Glu,
R_{32} is either Lys or Orn and the side chains thereof are

linked by an amide bond and that when R_{32} is Glu, R_{29} is either Lys or Orn and the side chains thereof are linked by an amide bond; and provided further that D-Phe[11] can be substituted by another D-isomer amino acid, preferably a D-

5 isomer of a natural amino acid, such as D-Leu and more preferably one other than D-Cys; that Glu in the 31-position can be substituted by any D-amino acid, e.g. D-Glu, D-Asp, D-Arg, (imBzl)D-His, β-(2naphthyl)-D-Ala, etc., again preferably a D-isomer of a natural amino acid other

10 than Cys; and that the N-terminus can be shortened by 1 or 2 residues. One particularly preferred Ucn analog is (cyclo 29-32)[Lys[29], D-Glu[31], Glu[32]]-Ucn, with Ucn being either hUcn or rUcn; others are described in the Examples hereinafter. When N-terminally shortened by 7 to 10

15 residues, these Ucn analogs are effective antagonists.

Extensive synthesis and testing over the past 10+ years have shown that ligands for the CRF receptors can tolerate a number of changes in amino acid sequence of native r/hCRF which do not result in significant changes in

20 bioactivity, such as would be indicative of the resulting analog being no longer able to bind and/or activate CRF receptors, particularly CRF-R1. As a result of this extensive earlier work, it has been found that one, two, or three substitutions can be made in the Ucn amino acid

25 sequence, within certain limits as set forth hereinafter, that will result in Ucn analogs which retain the bioactivity of Ucn and, in some instances, may have even more desirable pharmacological characteristics.

Using the amino acid sequence of Ucn as a reference,

30 these analogs should differ only by one, two or three substitutions from SEQ ID NO: 8 or from SEQ ID NO:15. The invention thus provides Ucn analogs according to the following amino acid sequence (SEQ ID NO: 14):

Y-Xaa$_1$-Xaa$_2$-Pro-Xaa$_4$-Xaa$_5$-Ser-Xaa$_7$-Asp-Leu-Xaa$_{10}$-Xaa$_{11}$-Xaa$_{12}$-

35 Xaa$_{13}$-Leu-Arg-Xaa$_{16}$-Xaa$_{17}$-Xaa$_{18}$-Xaa$_{19}$-Xaa$_{20}$-Xaa$_{21}$-Xaa$_{22}$-Xaa$_{23}$-
Xaa$_{24}$-Xaa$_{25}$-Xaa$_{26}$-Xaa$_{27}$-Xaa$_{28}$-Xaa$_{29}$-Ala-Xaa$_{31}$-Xaa$_{32}$-Asn-Arg-Xaa$_{35}$-
Xaa$_{36}$-Xaa$_{37}$-Xaa$_{38}$-Xaa$_{39}$-Xaa$_{40}$-NH$_2$, wherein Y is an acyl group

having 7 or less carbon atoms or hydrogen; Xaa_1 is Asp, Glu or Gln; Xaa_2 is Asn, Asp, Glu or Gly; Xaa_4 is Ser or Pro; Xaa_5 is Leu, Ile or Met; Xaa_7 is Ile or Leu; Xaa_{10} is Thr or Ser; Xaa_{11} is Phe or Leu; Xaa_{12} is His or Glu; Xaa_{13} is Leu

5 or Met; Xaa_{16} is Thr, Asn, Glu, or Lys; Xaa_{17} is Leu, Met or Val; Xaa_{18} is Leu or Ile; Xaa_{19} is Glu or His; Xaa_{20} is Leu, Met, Ile or Arg; Xaa_{21} is Ala, Glu or Thr; Xaa_{22} is Arg or Lys; Xaa_{23} is any natural amino acid and preferably Thr, Ser, Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu

10 or Asp; Xaa_{24} is Gln, Glu or Asp; Xaa_{25} is any natural amino acid and preferably Ser, Thr, Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{26} is Gln, Leu or Glu; Xaa_{27} is Arg, Ala or Lys; Xaa_{28} is Glu or Gln; Xaa_{29} is Arg or Gln; Xaa_{31} is any natural amino acid and preferably Ala,

15 Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{32} is any natural amino acid and preferably Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{35} is Ile, Lys, Leu or Asn; Xaa_{36} is Ile, Tyr, Met or Leu; Xaa_{37} is Phe, Leu or Met; Xaa_{38} is Asp or Glu; Xaa_{39} is Ser,

20 Ile, Glu or Thr; and Xaa_{40} is Val, Ile, Phe or Ala; provided that there are no more than 3 residues different from the hUcn or the rUcn amino acid sequence and that the N-terminus may be shortened by 1 or 2 residues. Moreover, these Ucn analogs when N-terminally shortened by 7 to 10

25 residues constitute effective antagonists.

The Ucn-like peptides of the invention may be chemically synthesized by any suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical

30 solution addition.

Ucn may also be synthesized by recombinant DNA techniques as may its analogs which include only natural amino acids. The amino acid sequence for rUcn (SEQ ID NO:8) was deduced from a partial cDNA clone isolated from a

35 rat brain cDNA library. Set forth in Table 2 hereinafter is the native rat nucleic acid sequence encoding Ucn (SEQ ID NO:9). The additional codon encoding glycine that is

present at the end of the native sequence is expected to account for the C-terminal amidation of rUcn.

TABLE 2

```
 5 GAC GAC CCG CCG TTG TCC ATC GAC CTC ACC TTC CAC CTG CTG CGG ACC
   Asp Asp Pro Pro Leu Ser Ile Asp Leu Thr Phe His Leu Leu Arg Thr
     1                   5               10                  15

   CTG CTA GAG CTA GCT CGG ACA CAG AGC CAG CGC GAG CGC GCA GAG CAG
10 Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln
                       20              25                  30

   AAC CGC ATC ATA TTC GAT TCG GTG GGCAAGTGA
   Asn Arg Ile Ile Phe Asp Ser Val
15               35                  40
```

Using the nucleic acid encoding rUcn as a probe, the
20 nucleic acid encoding the mature hUcn was isolated from a human genomic placental library. Set forth in TABLE 2A hereinafter is the portion of native human nucleic acid sequence encoding the mature Ucn peptide (see SEQ ID NO:16), with the additional codon for glycine at the end
25 being expected to account for C-terminal amidation.

TABLE 2A

```
   GAC AAC CCT TCT CTG TCC ATT GAC CTC ACC TTT CAC CTG CTG CGG ACC
   Asp Asn Pro Ser Leu Ser Ile Asp Leu Thr Phe His Leu Leu Arg Thr
30  1                   5               10                  15

   CTG CTG GAG CTG GCG CGG ACG CAG AGC CAG CGG GAG CGC GCC GAG CAG
   Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln
35                     20              25                  30

   AAC CGC ATC ATA TTC GAC TCG GTG GGCAAGTGA
   Asn Arg Ile Ile Phe Asp Ser Val
   35                  40
```

40

Synthesis by the use of recombinant DNA techniques, for purposes of this application, should be understood to include the suitable employment isolated nucleic acid encoding for Ucn or an appropriate analog, as is well known
45 in the art at the present time. As explained in detail hereinafter, synthetic Ucn peptides may be obtained by transforming a microorganism using an expression vector

including appropriate regulatory sequences associated with nucleic acid encoding a Ucn-like peptide and causing such transformed microorganism to express the Ucn peptide.

Because of the relative shortness of the Ucn-like

5 peptides, about 40 residues or less, chemical or chain elongation synthesis is presently felt to be the method of choice. Analogs of hUcn or rUcn having one or more substitutions can be readily synthesized in this manner and then tested for biological activity in a straightforward

10 manner to determine the specific biological effect of such substitution(s). Common to such chemical syntheses of peptides is the protection of the labile side chain groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from

15 occurring at that site until the group is ultimately removed. Also common is the protection of an alpha-amino group on an amino acid or a short peptide fragment while that entity reacts at the free carboxyl group to effect chain elongation, followed by the selective removal of the

20 alpha-amino protecting group to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with

25 various of these residues having side-chain protecting groups.

Thus, in such chemical syntheses, intermediates are formed having a protected amino acid sequence such as the following which is based on hUcn SEQ ID NO:15 (Formula II):

30 X^1-Asp(X^5)-Asn(X^4)-Pro-Ser(X^2)-Leu-Ser(X^2)-Ile-Asp(X^5)-Leu-Thr(X^2)-Phe-His(X^6)-Leu-Leu-Arg(X^3)-Thr(X^2)-Leu-Leu-Glu(X^5)-Leu-Ala-Arg(X^3)-Thr(X^2)-Gln(X^4)-Ser(X^2)-Gln(X^4)-Arg(X^3)-Glu(X^5)-Arg(X^3)-Ala-Glu(X^5)-Gln(X^4)-Asn(X^4)-Arg(X^3)-Ile-Ile-Phe-Asp(X^5)-Ser(X^2)-Val-X^7 (or suitably N-terminally

35 shortened versions thereof) wherein:

-22-

X^1 is either hydrogen or an alpha-amino protecting group. The alpha-amino protecting groups contemplated by X^1 are those known to be useful in the art in the step-wise synthesis of polypeptides. Among the classes of
5 alpha-amino protecting groups covered by X^1 are (1) acyl-type protecting groups, such as formyl, acrylyl(Acr), benzoyl(Bz) and acetyl(Ac) which are preferably used only at the N-terminal; (2) aromatic urethan-type protecting groups, such as benzyloxycarbonyl(Z) and substituted Z,
10 such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl; (3) aliphatic urethan protecting groups, such as t-butyloxycarbonyl (BOC), diisopropylmethoxycarbonyl, isopropyloxycarbonyl,
15 ethoxycarbonyl, allyloxycarbonyl; (4) cycloalkyl urethan-type protecting groups, such as fluorenylmethyloxycarbonyl(FMOC), cyclopentyloxycarbonyl, adamantyloxycarbonyl,and cyclohexyloxycarbonyl; and (5) thiourethan-type protecting groups, such as
20 phenylthiocarbonyl. The preferred alpha-amino protecting group is BOC if the synthesis employs acid-catalyzed removal of the alpha-amino protecting groups; however, for syntheses employing a base-catalyzed removal strategy, FMOC is preferred, in which case, more acid-labile side chain
25 protecting groups can be used, including t-Butyl esters or ethers as well as BOC.

X^2 is hydrogen or a protecting group for the hydroxyl group of Thr and Ser and is preferably acetyl(Ac), benzoyl(Bz), tert-butyl, triphenylmethyl(trityl),
30 tetrahydropyranyl, benzyl ether(Bzl) or 2,6-dichlorobenzyl (DCB). The most preferred protecting group is Bzl.

X^3 is hydrogen or a protecting group for the guanidino group of Arg, preferably selected from nitro, p-toluenesulfonyl(Tos), Z, adamantyloxycarbonyl and BOC.
35 Tos is preferred for a BOC strategy, and 4-methoxy-2,3,6-

-23-

trimethyl benzenesulfonyl (MTR) or pentamethyl chroman-6-sulfonyl (PMC) is preferred for FMOC strategies.

X^4 is hydrogen or a protecting group for the side chain amido group of Asn or Gln, preferably xanthyl(Xan).

5 Asn or Gln is preferably coupled without side chain protection in the presence of hydroxybenzotriazole (HOBt).

X^5 is hydrogen or an ester-forming protecting group for the β- or γ-carboxyl group of Asp or Glu, preferably cyclohexyl (OChx), benzyl (OBzl), 2,6-dichlorobenzyl,

10 methyl, ethyl and t-butyl ester (Ot-Bu). Chx is preferred for a BOC strategy and Ot-Bu for FMOC strategy.

X^6 is hydrogen or a protecting group for the side chain imidazole nitrogen of His, such as Tos.

The selection of a side chain amino protecting group

15 is not critical except that the protecting group should be one which is not removed during deprotection of the alpha-amino groups during the synthesis. Hence, the alpha-amino protecting group and the side-chain-amino protecting group cannot be the same.

20   X^7 is NH_2, a protecting group, such as an ester, or is an anchoring bond of the type used in solid phase synthesis for linking the peptide being synthesized to a solid resin support, preferably one represented by the formulae:

        -NH-benzhydrylamine (BHA) resin support and

25   -NH-paramethylbenzhydrylamine (MBHA) resin support.

Cleavage from a BHA or MBHA resin directly gives the Ucn-like peptide in amidated form. By employing a methyl-derivative of such a resin, if desired, the

30 corresponding, equivalent, methyl-substituted amide can be created. Alternatively, using an appropriate resin support, the ethylamide, which is also considered to be an equivalent, can be created as well known in this art.

In the formula for the intermediate, at least one of

35 X^1, X^2, X^3, X^4, X^5 and X^6 is a protecting group. In

selecting a particular side chain protecting group to be
used in the synthesis of the peptides, the following rules
are followed: (a) the protecting group should be stable to
the reagent, and under the reaction conditions, selected
5 for removing the alpha-amino protecting group at each step
of the synthesis, (b) the protecting group should retain
its protecting properties and not be split off under
coupling conditions and (c) the protecting group must be
removable upon completion of the synthesis under reaction
10 conditions that will not alter the peptide chain.

For the acyl group at the N-terminus of a Ucn-like
agonist peptide, which is represented by Y, acetyl,
formyl, acrylyl and benzoyl are preferred. Moreover, as
indicated earlier, the N-terminus can be slightly shortened
15 by removal of the N-terminal residue or the first two N-
terminal residues without significantly affecting
biological potency of the peptide to function as a Ucn
agonist, and when such shortening occurs, acylation of the
residue at the shortened N-terminus may be preferred. More
20 extensive shortening of the N-terminus by a sequence of 7
to 11 residues results in the creation of Ucn antagonists
which strongly bind CRF-R without activating the receptor
as discussed hereinafter.

Overall, there is broadly provided a process for the
25 manufacture of peptides defined by Formula I or analogs
thereof comprising (a) forming an intermediate peptide
according to Formula II or an analog thereof wherein there
is at least one protective group, with X^1, X^2, X^3, X^4, X^5 and
X^6 being each either hydrogen or a protective group, and X^7
30 being either a protective group or an anchoring bond to
resin support or NH_2 and (b) splitting off the protective
group or groups or anchoring bond from the intermediate
peptide of the Formula II and (c) if desired, converting
the resulting peptide into a nontoxic salt thereof.

When the peptides of the invention are prepared by
chemical synthesis, they are preferably prepared using
solid phase synthesis, such as that described by
Merrifield, J. Am. Chem. Soc., 85, p 2149 (1964), although
5 other equivalent chemical syntheses known in the art can
also be used as previously mentioned. Thus, Ucn-like
peptides can be prepared in a straightforward manner and
then simply tested for biological activity. This
facilitates the ready preparation and evaluation of Ucn-
10 like peptides which are analogs of hUcn or rUcn.
Solid-phase synthesis is preferably commenced from the
C-terminus of the peptide by coupling a protected
alpha-amino acid to a suitable resin as generally set forth
in U.S. Patent No. 4,244,946 issued Jan. 21, 1981 to Rivier
15 et al. by coupling with the free carboxyl group. The
synthesis of Ucn can be initiated by coupling
alpha-amino-protected Val to a BHA resin using methylene
chloride and dimethylformamide(DMF). Following the
coupling of BOC-Val to the resin support, the alpha-amino
20 protecting group may be removed using trifluoroacetic
acid(TFA) in methylene chloride, TFA alone or with HCl in
dioxane. Preferably 50 volume % TFA in methylene chloride
is used with 0-5 weight % 1,2 ethanedithiol. The
deprotection is carried out at a temperature between about
25 0°C and room temperature. Other standard cleaving reagents
and conditions for removal of specific alpha-amino
protecting groups may be used as described in Schroder &
Lubke, "The Peptides", 1, pp 72-75 (Academic Press 1965).
After removal of the alpha-amino protecting group of
30 Val, the remaining alpha-amino- and side chain-protected
amino acids are coupled stepwise in the desired order to
obtain the intermediate compound of Formula II. As an
alternative to adding each amino acid separately in the
synthesis, some of them may be coupled to one another prior
35 to addition to the solid phase reactor. The selection of

an appropriate coupling reagent is within the skill of the
art. Particularly suitable as coupling reagents are
N,N'-dicyclohexyl carbodiimide(DCCI) and N,N'-diisopropyl
carbodiimide(DICI).

5   Activating reagents used in the solid phase synthesis
of the peptides of the invention are well known in the
peptide art. Examples of suitable activating reagents are
carbodiimides, such as N,N'-diisopropyl carbodiimide and
N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide. Other
10 activating reagents and their use in peptide coupling are
described by Schroder & Lubke, supra, in Chapter III and by
Kapoor, J. Phar. Sci., 59, pp 1-27 (1970). P-nitrophenyl
ester (ONp) may also be used to activate the carboxyl end
of Asn or Gln for coupling. For example, BOC-Asn (ONp) can
15 be coupled overnight using one equivalent of HOBt in a 50%
mixture of DMF and methylene chloride, in which case no
DCCI is added.

Each protected amino acid or amino acid sequence is
introduced into the solid phase reactor in about a fourfold
20 excess, and the coupling is carried out in a medium of
dimethylformamide(DMF):CH_2Cl_2 (1:1) or in DMF or CH_2Cl_2
alone. In instances where the coupling is carried out
manually, the success of the coupling reaction at each
stage of the synthesis is monitored by the ninhydrin
25 reaction, as described by E. Kaiser et al., Anal. Biochem,
34, 595 (1970). In cases where incomplete coupling occurs,
the coupling procedure is repeated before removal of the
alpha-amino protecting group prior to the coupling of the
next amino acid. The coupling reactions can be performed
30 automatically, as on a Beckman 990 automatic synthesizer,
using a program such as that reported in Rivier et al.,
Biopolymers, 1978, 17, pp.1927-1938.

After the desired amino acid sequence has been
completed, the intermediate peptide is removed from the
35 resin support by treatment with a suitable clearing agent,

-27-

such as liquid hydrogen fluoride, which not only cleaves
the peptide from the resin but also cleaves all remaining
side chain protecting groups X^2, X^3, X^4, X^5 and X^6 and the
alpha-amino protecting group X^1 (unless it is an acyl group
5 which is intended to be present in the final peptide). When
using hydrogen fluoride for cleaving, anisole or cresole
and methylethyl sulfide are preferably included in the
reaction vessel as scavengers. The BOC protecting group at
the N-terminus is preferably cleaved with trifluoroacetic
10 acid(TFA)/ethanedithiol prior to the cleaving of the
peptide from the resin.

The determination of whether any Ucn-like peptide of
about 40 residues in length, or a fragment thereof, or an
antagonist version thereof, will have desirable
15 pharmacological properties can be made in a straightforward
manner. First, assays are run to determine the effect of a
candidate agonist or antagonist peptide on the different
CRF receptors; then, the ability of the peptide to promote
or inhibit production of ACTH is determined. Fragments
20 which function as CRF-BP blockers can be likewise similarly
assayed using an inhibitory binding assay with hCRF-BP and
a known labelled ligand.

The candidate peptide is easily evaluated in binding
assays with the various CRF receptors earlier discussed
25 using assays as described in Perrin, M., et al.,
Endocrinology, 118, 1171-1179 (1986). A binding assay with
human CRF-R1 is preferably carried out using a radioligand
oCRF analog; such a binding assay utilizing CRF-R1 receptor
is described in Chen, et al., *P.N.A.S., 90, supra*.
30   A straightforward assay using rat anterior pituitary
cells in monolayer culture can be carried out to determine
whether a candidate peptide thereof will function as a CRF
agonist and stimulate ACTH secretion by activating CRF
receptors on such cells. The procedure which is used is
35 that generally set forth in *Endocrinology, 91, supra*. A

very similar assay is used to test for antagonistic
properties, using a challenge dose of oCRF or the like.

By the *in vivo* administration to mammals of peptides
which have a high affinity to human CRF binding protein and
5 which thus compete with endogenous CRF and Ucn for binding
to hCRF-BP, CRF-BP is effectively blocked. This leaves
endogenous CRF and Ucn available in higher concentrations
to carry out their usual biological functions throughout
the body, particularly in localized areas where the peptide
10 is administered and/or where CRF-BP is present.

More specifically, fragments of Ucn, or analogs of
Ucn, between about 19 and 28 residues in length have a very
high affinity to hCRF-BP, but generally exhibit relatively
low propensity for binding CRF receptors. As a result,
15 these blocking fragments can be administered to prevent the
clearance of endogenous CRF and/or Ucn from particular
regions in the body and thereby stimulate the biological
effect of CRF and/or Ucn *in vivo*. In certain instances, it
may be advantageous to administer such peptides along with
20 CRF or Ucn or an agonist thereof. The very nature of these
fragments is such that potentially undesirable immunogenic
side effects are minimized or totally obviated. They might
also be administered along with antagonists to prevent the
clearance of antagonists having a fairly high binding
25 affinity to hCRF-BP from a target region. These blocking
fragments are useful for therapeutic treatment to promote
parturition in pregnancy, to stimulate the respiratory
system, to combat obesity, and to counteract the effects of
Alzheimer's disease, and of chronic fatigue syndrome; for
30 the latter four indications, the blocking fragments are
preferably administered in a manner so as to be delivered
to the brain.

Ucn peptides can be used in diagnostic methods to
detect the level of Ucn present in a body sample as well as
35 in an inoculum for the preparation of antibodies that

immunoreact with epitopes on Ucn. Antibodies generated
against Ucn can be employed for diagnostic applications,
therapeutic applications, and the like. Such antibodies
can be prepared employing standard techniques, as are well
5 known to those of skill in the art, using Ucn or a fragment
thereof, as an antigen. Antibodies of the present
invention are typically produced by immunizing a mammal,
e.g. rabbit, sheep, goat, etc., with an inoculum containing
Ucn or fragment thereof thereby inducing the production of
10 antibody molecules having immunospecificity for the
immunizing agent.

Antibodies which recognize Ucn are raised against
either the entire 40-residue amino acid sequence or against
a synthetic fragment of a sequence of at least about 5 or
15 preferably 6 residues. For example, such antibodies can be
raised against the 6 N-terminal residues, or against the 6
C-terminal residues, or against an interior sequence, such
as the sequence embracing residues 18-23. Such antibodies
will bind to and thus can be employed to indicate the
20 presence of Ucn; they are therefore useful in assays.
Moreover, certain of these antibodies will bind to and
biologically inactivate Ucn, and such antibodies can be
administered to animals for the purpose of neutralizing
endogenous Ucn. In an instance where endogenous antibodies
25 are being created that would bind Ucn, short amino acid
sequences from Ucn might be administered as antibody
blockers. To generate such blocking antibodies, either the
entire 40-residue sequence can be used, or a short peptide
sequence can be synthesized constituting one region of
30 particular interest. Such a synthetic short chain peptide
is generally conjugated to a large carrier molecule, and
the conjugate is then used as inoculum to induce a
mammalian immune system in rabbits or sheep or the like.
Details of the production of such polyclonal antibodies are
35 set forth in U.S. Patent No. 4,864,019 (9/5/89). If

-30-

instead of polyclonal antibodies, it is desired to produce
monoclonal antibodies, such can be made in a
straightforward manner using similar inoculum by employing
hybridoma techniques now well established in this art.

5 Details of exemplary monoclonal antibody production are set
forth in U.S. Patent No. 5,032,521 (7/16/91), and U.S.
Patent No. 5,051,364 (9/24/91).

Antibodies so produced can be used in diagnostic
methods and systems to detect the level of Ucn present in a
10 human or other mammalian body sample, such as tissue or
fluid. The anti-Ucn antibodies may also be used for
immunoaffinity or affinity chromatography purification of
Ucn, the details of which are well known in this art. In
addition, an anti-Ucn antibody can be used in human
15 therapeutic methods. Moreover, it is contemplated that DNA
encoding such antibodies may be injected via gene therapy
methods to raise desired antibodies within a patient or
alternatively to provide antibody blockers in an
appropriate situation.

20   The lack of evolutionary digression with respect to
the amino acid sequences of the 41-residue CRF biological
messenger in rats and humans (which sequences are
identical) is fairly indicative of the probability of
conserved regions in the corresponding amino acid sequences
25 of Ucn in mammalian species, such as human, bovine,
porcine, ovine, caprine, murine, canine, feline, baboon,
monkey, rabbit, etc. The corollary is that, once one has a
significant portion of the Ucn nucleic acid sequence of one
mammalian species, i.e. the rat sequence as disclosed
30 herein, it is a straightforward exercise to obtain
naturally occurring variant homolog nucleic acid sequences
of other animal species which encode homologs, such as CRF-
binding proteins (see e.g., Potter et al., Nature, 349,
423-426 (1991), where it was shown that the cDNA coding
35 region for human serum-derived CRF binding protein was of

-31-

sufficient homology in the rat cDNA coding region to permit
identification of the latter. The first disclosed nucleic
acid sequence (SEQ ID NO: 9) is of the native rat species;
in addition to its being useful in an expression vector to
5 express a Ucn peptide, it is also useful to obtain the DNA
of other mammalian species encoding the respective
counterpart Ucn peptides. In this respect, either the
entire nucleic acid sequence or nucleic acid sequences at
least about 14 nucleotides in length can be used as
10 hybridization probes to obtain and clone counterpart
mammalian sequences, as is presently well known in this
art. Similarly, primers based upon the foregoing nucleic
acid sequence can be used along with PCR (Polymerase Chain
Reaction) techniques to amplify nucleic acid sequences of
15 other mammalian species using suitable sources of DNA.
As used herein, a nucleic acid "probe" may be a
single-stranded DNA or RNA that has a nucleotide sequence
of at least 14, and preferably at least 20 or more,
contiguous bases that are the same as (or the complement
20 of) any 14 or more contiguous bases set forth in SEQ ID
NO:9.

Labeled nucleic acid encoding Ucn, or fragments
thereof, can be employed to probe cDNA libraries, genomic
libraries and the like for additional nucleotide sequences
25 encoding other novel mammalian members of the Ucn family.
Such screening may be initially carried out under
stringency conditions employing a temperature of about
42.5°C, a formamide concentration of about 20%, and a salt
concentration of about 5X standard saline citrate (SSC; 20X
30 SSC contains 3M sodium chloride, 0.3M sodium citrate, pH
7.5). Such conditions will allow the identification of
sequences having substantial similarity with the probe
sequence, without requiring perfect homology. By
"substantial similarity" is meant nucleotide sequences
35 which share at least about 50% homology. It may be

desirable to select hybridization conditions which will identify only sequences having at least 70% homology with the probe, while discriminating against sequences which have a lower degree of homology with the probe; such is

5 effected by increasing the stringency used to exceed the above-stated conditions as is well known in this art.

For example, using established methods well known to those skilled in the art (see e.g., *Molecular Cloning, A Laboratory Manual* 2Ed, Chapter 8, Construction and Analysis

10 of cDNA Libraries, J. Sambrook et al. (1989)), either the entire SEQ ID NO:9 or portions thereof of at least about 14 or 17 or 20 nucleotides in length may be used to screen mammalian genomic or cDNA libraries to identify and isolate homologous nucleic acids encoding Ucn from human and other

15 mammalian species. Oligonucleotide sequences of about 14 or 17 or 20 nucleotides or longer can be prepared by conventional *in vitro* synthesis techniques. Screening with such oligonucleotides as probes is preferably carried out under high stringency conditions as defined in Sambrook et

20 al., supra, Chapter 11, pp. 11.45-11.57.

As indicated above, possession of a native DNA sequence encoding a specific peptide hormone of one mammalian species allows one to obtain the homologous DNA sequence of other mammalian species. Isolation of

25 nucleotide sequences encoding Ucn peptides of other species often involves utilization of either a genomic library or a cDNA library made from RNA isolated from tissue containing Ucn. If such a source is available, it will generally be preferable to create a cDNA library for isolation of

30 nucleotide sequences encoding Ucn so as to avoid any possible problems arising from attempts to determine intron/exon borders. Libraries can be made in either eukaryotic or prokaryotic host cells. Widely available cloning vectors, such as plasmids, cosmids, phage and the

35 like, can be used to generate genetic libraries suitable

for the isolation of nucleotide sequences encoding Ucn peptides.

Methods for screening genetic libraries for the presence of target nucleotide sequences include using such
5 probes based on the sequence of a known nucleotide sequence are described in detail in Chapter 11 of Sambrook et al., supra. In the present situation, it may be preferable to use the entire length of the rat nucleotide sequence SEQ ID NO:9 labeled with radionuclides, enzymes, biotin,
10 fluoresces, or the like, as a probe for screening such genetic libraries.

Hybridization refers to the binding of complementary strands of nucleic acid (i.e., sense:antisense strands or probe:target-DNA) to each other through hydrogen bonds,
15 similar to the bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target-nucleic acid can be readily varied by those of skill in the art. As used herein, the phrase "high stringency" hybridization refers to conditions that permit
20 target-nucleic acid to bind a complementary nucleic acid that has at least about 80% homology to the target-nucleic acid. An example of such stringency conditions would be conditions that are minimally equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS
25 at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C. Denhart's solution and SSPE are desirable in Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989) and are well known to those of skill in this art; there are other
30 suitable hybridization buffers that may be used. It may be preferred to use stringency conditions requiring greater than about 90% homology to target-DNA.

As an example, the human form of Ucn was successfully cloned from a human placental genomic library.
35 Approximately 0.6 x 10^6 phage plaques of a human placental

genomic library in the EMBL3 SP6/T7 vector (Clontech) were
screened by hybridization using a probe corresponding to
the mature peptide region of rat Ucn. The 160 bp probe
encoding the rat Ucn mature peptide was synthesized by PCR
5 using the following oligos (sense: 5'-
TGCAGGCGAGCGGCAACGACGAGACGA-3') and (antisense: 5'-
ATACGGGGCCGATCACTTGCCCACCGAG-3') and [α^{32}P-dCTP].
Hybridization was carried out at 42°C in standard buffers
with 20% formamide. Final washes were at 42°C in 2X
10 SSC/0.1% SDS. The phage DNA from an individual positive
plaque for the clone was purified and then subcloned into
pBluescript (Strategene). Dideoxy sequencing was done
using the Sequenase kit (US Biochemical).

The genomic clone isolated from the library which
15 contains the gene for human Ucn has an insert size of
approximately 15 kb. A large number of base pairs of the
insert have been sequenced (see SEQ ID NO:16) in the region
corresponding to the precursor and the mature peptide
region of human Ucn. The portion of nucleotide sequence
20 initially sequenced encoding the mature human Ucn peptide,
as compared to the mature rat Ucn peptide, was set forth in
TABLE 2A hereinbefore. The nucleotide sequence for the
mature human Ucn peptide is 88% similar to the nucleotide
sequence for the rat Ucn peptide. The amino acid sequence
25 encoded by this region shows 95% similarity between human
Ucn and rat Ucn. The mature human peptide (see residues
83-122 of SEQ ID NO:15) is 40 residues of the total of 124.

Another suitable technique which may be used in the
present situation involves the use of primers based on
30 sequences derived from rat Ucn nucleic acid and the
polymerase chain reaction (PCR) to amplify target nucleic
acid. The target can then be isolated using a specific
hybridization probe based on the amplified segment, which
is then analyzed for its overall sequence and the
35 polypeptide which it encodes.

To synthesize a peptide of the invention by
recombinant DNA, a double-stranded DNA which encodes the
desired peptide can be synthetically constructed. Although
PCR techniques might nowadays be the method of choice to
5 produce a DNA sequence, for example, SEQ ID NO:9, DNA
encoding Ucn can be designed using certain codons that are
particularly efficient for polypeptide expression in a
certain organism, i.e. selection might employ those codons
which are most efficient for expression in the type of
10 organism which is to serve as the host for the recombinant
vector. However, any correct set of codons will encode a
desired product, although perhaps slightly less
efficiently. Codon selection may also depend upon vector
construction considerations; for example, it may be
15 necessary to avoid placing a particular restriction site in
the DNA coding sequence if, subsequent to inserting the
coding sequence, the vector is to be manipulated using a
restriction enzyme that cleaves at such a site. Of course
one would avoid placing restriction sites in the DNA coding
20 sequence if the host organism, which is to be transformed
with the recombinant vector, is known to produce a
restriction enzyme that would cleave at such a site within
the DNA chain.

Isolated nucleotide sequences encoding Ucn and analogs
25 thereof can be used to produce purified Ucn by either
recombinant DNA methodology or by in vitro polypeptide
synthesis techniques. The term "isolated" refers to a
nucleotide sequence or a polypeptide sequence that has been
manually produced and is separated from its native, in
30 vivo, cellular environment and is present in the
substantial absence of other biological molecules of the
same type. As a result of this human intervention, the
recombinant, isolated and/or substantially pure DNAs, RNAs,
polypeptides and proteins of the invention can be produced
35 in large quantities and are useful in ways that the DNAs,

RNAs, polypeptides or proteins as they naturally occur are
not, such as identification of selective drugs or
compounds. The term "purified" as used herein for
nucleotide sequences preferably means at least 95% by
5 weight, and most preferably at least 99% by weight, of
biological macromolecules of the same type present (but
water, buffers, and other small molecules, can be present).

To assemble a synthetic, nonchromosomal nucleic acid
sequence, oligonucleotides are constructed by conventional
10 procedures such as those described in Sambrook et al.,
supra. Sense and antisense oligonucleotide chains, up to
about 70 nucleotide residues long, are synthesized,
preferably on automated synthesizers well known in this
art. The oligonucleotide chains are constructed so that
15 portions of the sense and antisense oligonucleotides
overlap, associating with each other through hydrogen
bonding between complementary base pairs and thereby
forming double-stranded chains, in most cases with gaps in
the strands. Subsequently, the gaps in the strands are
20 filled in, and oligonucleotides of each strand are joined
end to end with nucleotide triphosphates in the presence of
appropriate DNA polymerases and/or with ligases. As an
alternative to such stepwise construction of a synthetic
nucleic acid sequence, DNA or cDNA encoding the complete
25 structure of a native polypeptide as obtained by screening
a library is used. As indicated hereinbefore,
amplification is preferably carried out by using PCR, and
the isolated and purified DNA is then incorporated into
recombinant molecules.
30 The desired nucleic acid coding sequence to be
inserted into a vector preferably has linkers at its ends
to facilitate insertion into restriction sites within the
cloning vector. Optionally, the nucleic acid coding
sequence may be constructed so as to encode the desired
35 peptide as a portion of a fusion polypeptide; and if so,

the coding sequence will generally contain terminal
sequences that encode amino acid residue sequences that
serve as proteolytic processing sites, whereby the encoded
polypeptide may be proteolytically cleaved from the
5 remainder of the fusion polypeptide. The terminal portions
of the nucleic acid coding sequence may also contain
appropriate start and stop signals.

The desired peptide is then expressed by recombinant
techniques after the nucleic acid coding sequence is
10 functionally inserted into a vector. By "functionally
inserted" is meant in proper reading frame and orientation,
as is well understood by those skilled in this art. For
example, when producing a genetic construction containing a
complete Ucn reading frame, the preferred starting material
15 is a cDNA library isolate encoding Ucn rather than a
genomic library isolate. Typically, the Ucn-encoding
sequence will be inserted downstream from a promoter and
will be followed by a stop codon, although production as a
hybrid protein followed by cleavage may be used, if
20 desired. In general, host-cell-specific sequences which
improve the production yield of Ucn will be used, and
appropriate control sequences will be added to the
expression vector, such as enhancer sequences,
polyadenylation sequences, and ribosome binding sites.
25   The production of Ucn can be carried out in both
prokaryotic and eukaryotic cell lines to provide protein
for biological and therapeutic use. While Ucn synthesis is
easily demonstrated using either bacteria or yeast cell
lines, the synthetic genes should also be insertable for
30 expression in cells of higher animals, such as Chinese
hamster ovary (CHO) cells or mammalian tumor cells as
described in detail in Sambrook et al, supra. Some
mammalian cells may be grown, for example, as peritoneal
tumors in host animals, and Ucn harvested from the
35 peritoneal fluid. Descriptions of mammalian expression

systems, baculovirus expression systems, bacterial
expression systems and yeast expression systems are set
forth in U.S. Patent No. 5,212,074 (5/18/93).

The following Examples set forth preferred chemical
5 methods for synthesizing Ucn, Ucn analogs, Ucn fragments,
and Ucn antagonists, by the solid-phase chain elongation
technique.

EXAMPLE I

10   The synthesis of rat Ucn having the formula (SEQ ID
NO:8):

Asp-Asp-Pro-Pro-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-
Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-
Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 is

15 conducted in a stepwise manner on a MBHA hydrochloride
resin, such as available from Bachem, Inc., having a
substitution range of about 0.1 to 0.5 mmoles/gm. resin.
The synthesis is performed on an automatic Beckman 990B
peptide synthesizer using a suitable program, such as the
20 following:

STEP	REAGENTS AND OPERATIONS	MIX TIMES MIN.
1	CH_2Cl_2 wash-80 ml. (2 times)	3
2	Methanol (MeOH) wash-30 ml. (2 times)	3
3	CH_2Cl_2 wash-80 ml. (3 times)	3
4	50 percent TFA plus 5 percent 1,2-ethanedithiol in CH_2Cl_2-70 ml. (2 times)	12
5	Isopropanol wash-80 ml. (2 times)	3
6	TEA 12.5 percent in CH_2Cl_2-70 ml. (2 times)	5
7	MeOH wash-40 ml. (2 times)	2
8	CH_2Cl_2 wash-80 ml. (3 times)	3

9	Boc-amino acid (10 mmoles in	30-300
	30 ml. of either DMF or CH_2Cl_2,	
	depending upon the solubility of	
	the particular protected amino	
	acid, (1 time), plus DCCI	
	(10 mmoles) in CH_2Cl_2	

Coupling of BOC-Val results in the substitution of about
5 0.35 mmol Val per gram of resin. All solvents that are
used are carefully degassed, preferably by sparging with an
inert gas, e.g. helium or nitrogen, to insure the absence
of oxygen.

After deprotection and neutralization, the peptide
10 chain is built step-by-step on the resin. Generally, one
to two mmol. of BOC-protected amino acid in methylene
chloride is used per gram of resin, plus one equivalent of
2 molar DCCI in methylene chloride, for two hours for the
coupling of each additional residue. When BOC-Arg(Tos) is
15 being coupled, a mixture of 50% DMF and methylene chloride
is used. Bzl is used as the hydroxyl side-chain protecting
group for Ser and Thr. The amido group of Asn or Gln can
be protected by Xan but need not be. BOC-Asn or BOC-Gln is
coupled overnight using one equivalent of DCC and two
20 equivalents of HOBt in a 50% mixture of DMF and methylene
chloride. Tos is used to protect the guanidino group of
Arg and the imidazole nitrogen of His. The side chain
carboxyl group of Glu or Asp is protected by OChx. At the
end of the synthesis, the following composition is
25 obtained:
BOC-Asp(OChx)-Asp(OChx)-Pro-Pro-Leu-Ser(Bzl)-Ile-Asp(OChx)-
Leu-Thr(Bzl)-Phe-His(Tos)-Leu-Leu-Arg(Tos)-Thr(Bzl)-Leu-
Leu-Glu(OChx)-Leu-Ala-Arg(Tos)-Thr(Bzl)-Gln-Ser(Bzl)-Gln-
Arg(Tos)-Glu(OChx)-Arg(Tos)-Ala-Glu(OChx)-Gln-Asn-Arg(Tos)-
30 Ile-Ile-Phe-Asp(OChx)-Ser(Bzl)-Val-NH-resin support.

In order to cleave and deprotect the resulting
peptide, the peptide-resin is treated with 1.5 ml. anisole,
0.5 ml. of methylethylsulfide and 15 ml. hydrogen fluoride

(HF) per gram of peptide-resin, first at -20°C. for 20 min. and then at 0°C for one and one-half hours. After elimination of HF under high vacuum, the resin-peptide is washed with dry diethyl ether, and the peptide amide is

5 then extracted with de-gassed 2N aqueous acetic acid or a 1:1 mixture of acetonitrile and water. The extract is separated from the resin by filtration, and then lyophilized.

The lyophilized peptide amide is purified by

10 preparative or semi-preparative HPLC as described in Rivier et al., J. Chromatography, 288, 303-328 (1984); and Hoeger et al., Biochromatography, 2, 3, 134-142 (1987). The chromatographic fractions are carefully monitored by HPLC, and only the fractions showing substantial purity are

15 pooled.

Specific optical rotation of the isolated and purified Ucn peptide is measured on a Perkin Elmer Model 241 Polarimeter as $[\alpha]_D = -62.5° \pm 1.0$ (c=1 in 1% acetic acid, without correction for the presence of H_2O and TFA); it has

20 a purity of greater than about 95%. Purity is further confirmed by mass spectroscopy and capillary zone electrophoresis (CZE). Liquid secondary ion mass spectrometry (LSIMS) mass spectra are measured with a JEOL model JMS-HX110 double-focusing mass spectrometer fitted

25 with a Cs^+ gun. An accelerating voltage of 10 kV and Cs^+ gun voltage between 25 and 30 kV are employed. The measured value of 4705.36 Da obtained using LSIMS is in agreement with the calculated value of 4705.52 Da.

To verify the precise sequence, the Ucn peptide is

30 hydrolyzed in sealed evacuated tubes containing constant boiling HCl, 3 μl of thioglycol/ml. and 1 nmol of Nle (as an internal standard) for 9 hours at 140°C. Amino acid analyses of the hydrolysates using a standard amino acid analyzer shows the expected amino acid ratios, which

35 confirms that a 40-residue peptide structure is obtained

with the expected amino acid residues which constitute the
intended sequence.

To provide a labelled Ucn peptide for use in assays,
including binding assays and the like, the synthesis is
5 extended to link Tyr to Asp at the N-terminus, producing
the 41-residue peptide referred to as [Tyr⁰]-Ucn, which can
be readily iodinated with ^{125}I and then used in diagnostic
assays and in drug-screening assays.

To test the ability of purified Ucn or another
10 candidate Ucn-like peptide to function as a CRF agonist and
to promote ACTH production and/or secretion, cultures of
dispersed primary murine anterior pituitary cells (0.15 x
10^6 cells/well are used), as generally described in Vale et
al. _Meth. Enzym., 103_, 565-577 (1983), and in
15 _Endocrinology, 91_, 562 (1972). These cultures are
maintained in 0.5 ml/well of β-PJ (a reagent available from
Kansas City Biochemicals) containing 2% fetal bovine serum.
On the morning of culture day 5, the cells are washed 3
times with β-PJ containing 0.1% bovine serum albumin and
20 then incubated for 1 hour at 37°C in the same medium. The
medium is then replaced with Ucn, or an analog thereof,
diluted in β-PJ containing 0.1% bovine serum albumin.
Incubations are terminated after 3 hours, at which time the
medium is removed and stored at -20°C until ACTH
25 radioimmunoassays are performed using a suitable kit, such
as that commercially available from Diagnostic Products
Corporation of Los Angeles, CA. Secreted ACTH is measured
using Allfit computer program with results expressed as the
average ± s.e.m. of 3 replicate bioassays.
30   The rUcn peptide strongly stimulates the secretion of
ACTH and β-endorphin in cultured rodent pituitary cells.
It is more biopotent than either r/hCRF or sauvagine,
having an EC_{50} of about 0.006 ± 0.003 nanomolar, compared
to about 0.043 ± 0.012 nM and about 0.033 ± 0.010 nM,
35 respectively; it is also more potent than oCRF. In

-42-

addition, it is more potent than suckerfishUI (sfUI) which
has an EC_{50} of 0.017 ± 0.003. rUcn also stimulates ACTH
and β-END-LI secretion *in vivo* in rats to a greater extent
than r/hCRF; in fact, at 30 minutes, a 1 μg/kg dose of rUcn

5   elevates ACTH level in plasma to a substantially greater
extent $(659 \pm 53$pg/ml) than does a 5 μg/kg dose of r/hCRF
$(422 \pm 66$ pg/ml), using an assay as generally described in
Science, 218, 377 (1982). Such a greater effect continues
at 1 hour and at 2 hours. The peptide when administered

10 peripherally, e.g. iv, also causes a marked fall in mean
arterial blood pressure in rats at a dose as low as 250 ng
for a standard laboratory rat of about 250 to 275 grams.
At a dose of about 3.77 μg/kg, it lowers blood pressure 2-3
times as much as either sfUI or r/hCRF and for a longer

15 duration.

Testing is also carried out for the ability of Ucn to
cause elevation of the level of intracellular cAMP in cells
which express murine CRF-R1 and also in cells which express
murine CRF-R2β using an assay as generally described in

20 Chen et al., Expression Cloning of a Human Coritcotropin
Releasing Factor (CRF) Receptor, *P.N.A.S., 90*, 8967-8971
(1993). rUcn is slightly more potent than either r/hCRF or
sfUI in elevating cAMP levels in cells expressing mCRF-R1.
However, the effect is even more dramatic in assays

25 utilizing cells expressing mCRF-R2β wherein the ED_{50} for
rUcn is 0.18 ± 0.04 nanomolar, compared to an ED_{50} for
r/hCRF of 1.7 ± 0.4 nM and an ED_{50} for urotensin of $0.74 \pm$
0.1 nM. It is also more potent than sauvagine which
exhibits an ED_{50} of 0.5 ± 0.2.

30   Binding assays with cells expressing human CRF-R1 are
carried out as described in the Chen et al. *P.N.A.S.,*
supra. The affinities of test peptides for CRF-R1 and CRF-
R2β stably expressed in CHO cells were determined by
competitive displacement of ^{125}I-(Nle^{21}, Tyr^{32}) ovine CRF

35 (for CRF-R1) or of [^{125}I-Tyr$^{\circ}$]Ucn (for CRF-R2β) as

described.   Data from at lest 3 experiments were pooled and
inhibitory dissociation constant (K_i) values (95%
confidence limits) were calculated using the LIGAND program
of Munson and Rodbard (1980), *Anal. Biochem,* 107:220-239.
5 The cloned hCRF-R1 binds Ucn with high affinity as
determined by the competitive displacement of bound
radioligand.   The K_i for rUcn was determined to be about
0.16(0.08 - 0.32)nM, compared to r/hCRF of about 0.95(0.47
- 2.0)nM, sfUI of about 0.43(0.23 - 0.81)nM, and sauvagine
10 of about 1.2(0.54 - 2.5)nM.   Again, the difference is even
more dramatic for similar stably transfected CHO cells
expressing human CRF-R2β where the respective results were
0.41(0.26 - 0.66)nM, 17(10 - 29)nM, 3.0(1.8 - 4.8)nM and
2.0(1.1 - 3.6)nM.   Testing also shows that rUcn binds more
15 strongly than does r/hCRF to human CRF binding protein
(hCRF-BP) by a factor of about 2, using a competitive hCRF-
BP ligand binding assay with the radioligand $^{125}I[Nle^{21}$,
$Tyr^{32}]$-oCRF, much more strongly than does sauvagine, but
less strongly than sfUI.
20

EXAMPLE IA

Human Ucn(1-40) having the formula (see SEQ ID NO:15):
H-Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-
Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-
25 Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 is
synthesized in the manner described in Example I.   LSIMS
shows a value of 4694.31 Da which agrees with the
calculated value of 4694.51 Da.   In vitro testing for ACTH
secretion using anterior pituitary cell cultures as set
30 forth in Example I shows that the peptide is about 3 times
as effective as r/hCRF, i.e. 3.10 (1.41 - 6.65).   The
peptide also has significant mammalian
vasodilatory-hypotensive activity, including lowering
systemic blood pressure and stimulating the secretion of
35 ACTH.

EXAMPLE IB

The peptide [Tyr°]rUcn(1-40) having the amino acid
sequence (see SEQ ID NO:8):
H-Tyr-Asp-Asp-Pro-Pro-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-
5 Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-
Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is
synthesized in the manner described in Example I.   LSIMS
shows a value of 4868.58 Da which agrees perfectly with the
calculated value of 4868.58 Da.   _In vitro_ testing for ACTH
10 secretion using anterior pituitary cell cultures as set
forth in Example I shows that the peptide is about twice as
effective as r/hCRF, i.e. 2.20 (1.28 - 3.88).   The peptide
also has significant mammalian vasodilatory-hypotensive
activity, including lowering systemic blood pressure and
15 stimulating the secretion of ACTH.

EXAMPLE IC

The peptide [D-Tyr°]hUcn(1-40) having the formula:
H-D-Tyr-Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-
20 Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-
Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$
is synthesized in the manner described in Example I.   LSIMS
shows a value of 4857.37 Da which agrees with the
calculated value of 4857.58 Da.   _In vitro_ testing for ACTH
25 secretion using anterior pituitary cell cultures as set
forth in Example I shows that the peptide is about 1.25
times as effective as r/hCRF, i.e. 1.23 (0.60 - 2.54).   The
peptide also has significant mammalian vasodilatory-
hypotensive activity, including lowering systemic blood
30 pressure and stimulating the secretion of ACTH.

EXAMPLE II

The peptide [Ac-Pro³]-hUcn(3-40) having the amino acid
sequence (see SEQ ID NO:15):

Ac-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-
Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-
Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is synthesized
in the manner described in Example I but, in addition, the
5 N-terminus is subjected to acetylation by treatment with
acetic anhydride after removal of the BOC-protecting group.
The resultant peptide likewise stimulates the secretion of
ACTH and β-END-LI and causes vasodilatory-hypotensive
activity, including lowering of systemic blood pressure.
10

EXAMPLE IIA

The peptide (cyclo 29-32) [Ac-Pro^3,D-Phe^{11}, Glu^{29}, D-
Glu^{31}, Lys^{32}]-hUcn(3-40) having the amino acid sequence:
Ac-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-Leu-Arg-
15 Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Glu-
Ala-D-Glu-Lys-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is
synthesized in the manner as generally described in Example
I but, in addition, the N-terminus is subjected to
acetylation by treatment with acetic anhydride after
20 removal of the BOC-protecting group. The cyclizing lactam
bond is created as described in Example I of U.S. Patent
No. 5,064,939. LSIMS shows a value of 4562.36 Da which
agrees with the calculated value of 4462.42 Da. _In vitro_
testing for ACTH secretion using anterior pituitary cell
25 cultures as set forth in Example I shows that the peptide
is about 6 times as effective as r/hCRF, i.e. 6.14 (2.83 -
14.05). The resultant peptide likewise stimulates the
secretion of ACTH and β-END-LI and causes
vasodilatory-hypotensive activity, including lowering of
30 systemic blood pressure.

EXAMPLE IIB

The peptide (cyclo 29-32) [Ac-Pro^3,D-Pro^4,D-Phe^{11},
Glu^{29}, Lys^{32}]-hUcn(3-40) having the amino acid sequence:

Ac-Pro-D-Pro^4-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-Leu-
Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-
Glu-Ala-Glu-Lys-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is
synthesized in the manner as generally described in Example

5 I but, in addition, the N-terminus is subjected to
acetylation by treatment with acetic anhydride after
removal of the BOC-protecting group. The cyclizing lactam
bond is created as described in Example I of U.S. Patent
No. 5,064,939. LSIMS shows a value of 4472.40 Da which

10 agrees with the calculated value of 4472.44 Da. _In vitro_
testing for ACTH secretion using anterior pituitary cell
cultures as set forth in Example I shows that the peptide
is about 10 times as effective as r/hCRF, i.e. 9.90 (4.48 -
22.85). The resultant peptide likewise stimulates the

15 secretion of ACTH and β-END-LI and causes
vasodilatory-hypotensive activity, including lowering of
systemic blood pressure.

EXAMPLE IIC

20   The peptide (cyclo 29-32) [Ac-Pro^3,D-Ser^4,D-Phe^{11},
Glu^{29}, Lys^{32}]-hUcn(3-40) having the amino acid sequence:
Ac-Pro-D-Ser-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-Leu-Arg-
Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Glu-
Ala-Glu-Lys-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is

25 synthesized in the manner as generally described in Example
I but, in addition, the N-terminus is subjected to
acetylation by treatment with acetic anhydride after
removal of the BOC-protecting group. The cyclizing lactam
bond is created as described in Example I of U.S. Patent

30 No. 5,064,939. LSIMS shows a value of 4462.33 Da which
agrees with the calculated value of 4462.42. _In vitro_
testing for ACTH secretion using anterior pituitary cell
cultures as set forth in Example I shows that the peptide
is about 5.75 times as effective as r/hCRF, i.e. 5.69 (2.43

35 - 14.49). The resultant peptide likewise stimulates the

secretion of ACTH and β-END-LI and causes vasodilatory-hypotensive activity, including lowering of systemic blood pressure.

5   **EXAMPLE III**

The peptide hUcn(2-40) having the amino acid sequence (see SEQ ID NO:15):

H-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-

10 Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 is synthesized in the manner described in Example I. The peptide has significant mammalian vasodilatory- hypotensive activity, including lowering systemic blood pressure and stimulating the secretion of ACTH.

15

EXAMPLE IV

The peptide [D-Phe[11]]-hUcn having the amino acid sequence: H-Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-

20 Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 is synthesized in the manner described in Example I. The peptide has significant mammalian vasodilatory-hypotensive activity, including lowering systemic blood pressure and stimulating the secretion of ACTH.

25

EXAMPLE V

The peptide agonist analogs of hUcn as set forth hereinafter, which are considered to have substantially the same amino acid sequence as hUcn, are synthesized:

30

[Glu[1]]-hUcn	[Leu[36]]-hUcn
[Ile[5]]-hUcn	[Ile[18]]-hUcn
[Val[17]]-hUcn	[Ile[20]]-hUcn
[Lys[22]]-hUcn	[Lys[27]]-hUcn
35 [Leu[35]]-hUcn	[Leu[37]]-rUcn

[Glu^{38}]-hUcn   [Ile^{40}]-rUcn

[Ser^{10}]-hUcn   [Thr^{39}]-rUcn

[Leu^{11}]-hUcn   [Leu^{7}]-rUcn

[Glu^{2}]-hUcn   [Leu^{7,11}]-rUcn

5   [Gln^{29}]-hUcn   [Lys^{22,27}]-rUcn

[Asn^{32}]-hUcn   [Ile^{5}, Gln^{29}]-rUcn

Each of the foregoing Ucn-like agonist peptides has
significant mammalian vasodilatory-hypotensive activity,
10 including lowering systemic blood pressure and stimulating
the secretion of ACTH.

EXAMPLE VI

A further group of Ucn-like agonist peptides are
15 synthesized which fall within the following amino acid
sequence:

Y-Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-
Leu-Arg-Thr-Leu-Leu-R$_{19}$-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-
Glu-R$_{29}$-Ala-Glu-R$_{32}$-Asn-Arg-Ile-R$_{36}$-Phe-R$_{38}$-Ser-Val-NH$_2$,

20 wherein Y is an acyl group having 7 or less carbon atoms or
hydrogen; R$_{19}$ is Glu or Ala; R$_{29}$ is Arg, Glu, Lys or Orn; R$_{32}$
is Gln, Lys, Orn or Glu; R$_{36}$ is Ile, C$^{\alpha}$MeIle or C$^{\alpha}$MeLeu; R$_{38}$
is Asp or Ala; provided that when R$_{29}$ is Glu, R$_{32}$ is either
Lys or Orn and the side chains thereof are linked by an
25 amide bond, and that when R$_{29}$ is either Lys or Orn, R$_{32}$ is
Glu, and the side chains thereof are linked by an amide
bond. In this group, D-Phe^{11} can be replaced by D-Leu or
by a D-isomer of another natural α-amino acid; Glu in the
31-position can be replaced by a D-isomer, e.g. D-Glu, D-
30 Arg, imBzlD-His, etc.; and the N-terminus can be shortened
by 1 or 2 residues.

The specific peptides are as follows:
[Ac-Pro^{3},D-Phe^{11}]-hUcn(3-40)
35   [D-Leu^{11}, Ala^{19}]-hUcn

[D-Phe^{11}, Ala^{38}]-hUcn

[D-Tyr^{11}, C$^{\alpha}$MeIle^{36}]-hUcn

[D-Phe^{11}, C$^{\alpha}$MeLeu^{36}]-hUcn

[Ac-Asp^{1}, D-Phe^{11}, Ala^{19,38}]-hUcn

5   (cyclo 29-32)[D-Leu^{11}, Glu^{29}, Lys^{32}]-hUcn

(cyclo 29-32)[D-Phe^{11}, Glu^{29}, Orn^{32}]-hUcn(2-40)

(cyclo 29-32)[Ac-Pro^{3},D-Phe^{11}, Lys^{29}, Glu^{32}]-hUcn(3-40)

(cyclo 29-32)[D-Tyr^{11}, Orn^{29}, Glu^{32}]-hUcn(3-40)

(cyclo 29-32)[D-Phe^{11}, Glu^{29}, D-Glu^{31}, Lys^{32}]-hUcn

10   (cyclo 29-32)[D-Phe^{11}, Glu^{29}, D-Arg^{31}, Lys^{32}]-hUcn

(cyclo 29-32)[D-Tyr^{11}, Glu^{29}, imBzD-His^{31}, Lys^{32}]-hUcn

Each of the foregoing Ucn-like agonist peptides has
significant mammalian vasodilatory-hypotensive activity,
15 including lowering systemic blood pressure and stimulating
the secretion of ACTH.

EXAMPLE VII

A still further group of Ucn-like agonist peptides
20 are synthesized which fall within the following amino acid
sequence (SEQ ID NO: 14):

Y-Xaa$_{1}$-Xaa$_{2}$-Pro-Xaa$_{4}$-Xaa$_{5}$-Ser-Xaa$_{7}$-Asp-Leu-Xaa$_{10}$-Xaa$_{11}$-Xaa$_{12}$-
Xaa$_{13}$-Leu-Arg-Xaa$_{16}$-Xaa$_{17}$-Xaa$_{18}$-Xaa$_{19}$-Xaa$_{20}$-Xaa$_{21}$-Xaa$_{22}$-Xaa$_{23}$-
Xaa$_{24}$-Xaa$_{25}$-Xaa$_{26}$-Xaa$_{27}$-Xaa$_{28}$-Xaa$_{29}$-Ala-Xaa$_{31}$-Xaa$_{32}$-Asn-Arg-Xaa$_{35}$-
25 Xaa$_{36}$-Xaa$_{37}$-Xaa$_{38}$-Xaa$_{39}$-Xaa$_{40}$-NH$_{2}$, wherein Y is an acyl group
having 7 or less carbon atoms or hydrogen; Xaa$_{1}$ is Asp, Glu
or Gln; Xaa$_{2}$ is Asn, Asp, Glu or Gly; Xaa$_{4}$ is Ser or Pro;
Xaa$_{5}$ is Leu, Ile or Met; Xaa$_{7}$ is Ile or Leu; Xaa$_{10}$ is Thr or
Ser; Xaa$_{11}$ is Phe or Leu; Xaa$_{12}$ is His or Glu; Xaa$_{13}$ is Leu
30 or Met; Xaa$_{16}$ is Thr, Asn, Glu, or Lys; Xaa$_{17}$ is Leu, Met or
Val; Xaa$_{18}$ is Leu or Ile; Xaa$_{19}$ is Glu or His; Xaa$_{20}$ is Leu,
Met, Ile or Arg; Xaa$_{21}$ is Ala, Glu or Thr; Xaa$_{22}$ is Arg or
Lys; Xaa$_{23}$ is any natural amino acid and preferably Thr,
Ser, Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu
35 or Asp; Xaa$_{24}$ is Gln, Glu or Asp; Xaa$_{25}$ is any natural amino

acid and preferably Ser, Thr, Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{26} is Gln, Leu or Glu; Xaa_{27} is Arg, Ala or Lys; Xaa_{28} is Glu or Gln; Xaa_{29} is Arg or Gln; Xaa_{31} is any natural amino acid and preferably Ala,

5 Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{32} is any natural amino acid and preferably Ala, Ile, Met, Val, Asn, Gln, Gly, Lys, His, Leu, Glu or Asp; Xaa_{35} is Ile, Lys, Leu or Asn; Xaa_{36} is Ile, Tyr, Met or Leu; Xaa_{37} is Phe, Leu or Met; Xaa_{38} is Asp or Glu; Xaa_{39} is Ser,

10 Ile, Glu or Thr; and Xaa_{40} is Val, Ile, Phe or Ala; provided that there are no more than 3 residues different from Ucn, and that the N-terminus may be shortened by 1 or 2 residues.

The following specific peptides are synthesized:

15 $[Ac-Pro^3, Met^5]-hUcn(3-40)$

$[Glu^{12}]-hUcn$ $[Met^{13}]-hUcn$

$[Asn^{16}, Ala^{23}]-hUcn$ $[Glu^{16}, Ala^{23,31}]-hUcn$

$[Lys^{16}, Ile^{23}, Ala^{32}]-hUcn$ $[Val^{17}, Ile^{23}, Met^{31}]-hUcn$

$[Met^{17}, Ile^{31,32}]-hUcn$ $[Ser^{10}, Ile^{18}, His^{19}]-hUcn$

20 $[Glu^1, Met^{20}, Lys^{35}]-hUcn$ $[Ile^{20}, Met^{32}, Glu^{38}]-hUcn$

$[Arg^{20}, Asn^{23}, Thr^{25}]-hUcn$ $[Thr^{21}, Gly^{23,32}]-hUcn$

$[Glu^{21}, Lys^{23}, Gln^{28}]-hUcn$ $[Lys^{22}, His^{23}, Leu^{31}]-hUcn$

$[Val^{23}, Glu^{24}, Met^{36}]-hUcn$ $[Gln^{23}, Asp^{25}, Glu^{39}]-hUcn$

$[Met^{23}, Gly^{25}, Leu^{35}]-hUcn$ $[Glu^{23}, Tyr^{36}, Phe^{40}]-hUcn$

25 $[Asp^{23}, Lys^{27}, Leu^{32}]-hUcn$ $[Ser^{23}, Asp^{24}, Met^{25}]-hUcn$

$[Ac-Pro^3, Leu^{23,25}, Gln^{29}]-hUcn(3-40)$

$[Asn^{25,32}, Ile^{39}]-hUcn$ $[Gln^{25,31}, Asn^{35}]-hUcn$

$[Lys^{25}, Val^{31}, Ala^{32}]-hUcn$ $[Glu^2, Asn^{31}, His^{32}]-hUcn$

$[Gln^1, Ile^5, Val^{32}]-hUcn$ $[Gly^2, Leu^{11}, Thr^{31}]-hUcn$

30 $[Glu^{25}, Leu^{37}]-hUcn$ $[Ile^5, His^{25}, Ala^{40}]-hUcn$

$[Leu^7, His^{31}, Asp^{32}]-hUcn$ $[Gln^{32}, Leu^{36}, Ala^{40}]-hUcn$

$[His^{25}, Ala^{31}, Thr^{39}]-hUcn$ $[Leu^{26}, Lys^{31}, Thr^{32}]-hUcn$

$[Glu^{26}, Ala^{27}, Lys^{32}]-hUcn$ $[Lys^{27}, Asp^{31}, Met^{37}]-hUcn$

$[Ac-Pro^3, Val^{25}, Leu^{36}]-hUcn(3-40)$

35 $[Ala^{25}, Asp^{31}, Ile^{40}]-hUcn$ $[Val^{32}, Leu^{37}, Ile^{39}]-hUcn$

[Leu^{11}, Ile^{18}, Thr^{39}]-hUcn

Each of the foregoing Ucn-like agonist peptides has significant mammalian vasodilatory-hypotensive activity,
5 including lowering systemic blood pressure and stimulating the production of ACTH.

The following group of Examples are directed to N-terminally shortened versions (e.g. shortened by 7-10 residues) of the Ucn-like peptides which have antagonistic
10 properties. All of the statements made hereinbefore with respect to the chemical character and/or the synthesis of Ucn analogs are considered to apply equally to the antagonists and are not thus repeated; the antagonists are merely N-terminally shortened versions of the agonists.
15 The specific peptides set forth in the following Examples exhibit antagonistic biological properties with respect to the effect of Ucn on at least the CRF receptors, CRF-R1, and CRF-R2. In this respect these Ucn-antagonists are considered to generally at least have characteristics and
20 uses similar to those described for CRF antagonists in U.S. Patent No. 5,245,009.

EXAMPLE VIII

The peptide, Ucn(11-40), having the amino acid
25 sequence (see SEQ ID NO:8):
H-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is synthesized in the manner described in Example I. To evaluate the biological activity of a
30 candidate peptide as a Ucn antagonist (which will be indicative of its effective binding to CRF receptors), the previously mentioned assay from *Endocrinology, 91, supra,* is run in the presence of a challenge dose of ovine CRF. The performance of such candidate in this assay is
35 routinely compared to the performance of a highly potent

linear CRF antagonist, i.e. [D-Phe[12], Nle[21,38]]-r/hCRF(12-41) which is hereinafter referred to as the Standard Antagonist. An *in vivo* test measuring elevation of mean arterial blood pressure as a result of *iv* injection is

5 also simply and straightforwardly performed. The peptide Ucn(11-40) exhibits significant mammalian vasoconstrictive activity causing elevation of mean arterial blood pressure, as do known CRF antagonists.

The synthesis is repeated twice to produce Ucn (10-
10 40) and [Ac-Thr[10]]Ucn(10-40); both show bioactivity as Ucn antagonists.

EXAMPLE IX

The peptide, [D-Phe[11]]-Ucn(11-40), having the amino
15 acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is synthesized in the manner described in Example I. Its specific optical rotation is measured
20 under the conditions set forth hereinbefore as $[\alpha]_D = -62°$ ± 1.0. LSIMS shows a value of 3638.88 Da which agrees with the calculated value of 3639.00 Da. <u>In vitro</u> testing is carried out as described in Example VIII, which demonstrates the peptide is bioactive, exhibiting a value
25 of 0.551 (0.333 - 0.857) compared to this highly biopotent Standard Antagonist. The peptide has significant mammalian vasoconstrictive activity, causing elevation of mean arterial blood pressure, indicative of its being a Ucn antagonist.

30

EXAMPLE X

The peptide, [D-Tyr[11]]-Ucn(11-40), having the amino acid sequence:
H-D-Tyr-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
35 Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-

Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
Example I. The peptide has significant mammalian
vasoconstrictive activity causing elevation of mean
arterial blood pressure, indicative of its being a Ucn
5 antagonist. It is also effectively iodinated to provide
[^{125}I-D-Tyr^{11}]-Ucn(11-40) for use in screening assays and
the like.

EXAMPLE XI

10   The peptide, (cyclo 29-32)[D-Phe^{11}, Glu^{29}, Lys^{32}]-
Ucn(11-40), having the amino acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
Gln-Ser-Gln-Arg-Glu-Glu-Ala-Glu-Lys-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
15 Example I, with the cyclizing lactam bond being created as
described in Example I of U.S. Patent No. 5,064,939,
issued Nov. 12, 1991. Its specific optical rotation is
measured under the conditions set forth hereinbefore as
[α]$_D$ = -49° ± 1.0. LSIMS shows a value of 3593.89 Da which
20 agrees with the calculated value of 3593.97 Da. _In vitro_
testing for ACTH secretion using anterior pituitary cell
cultures as set forth in Example VIII shows that the
peptide is about 10 times as effective as the Standard
Antagonist, i.e. 10.34 (4.27 - 25.58). The peptide also
25 has significant mammalian vasoconstrictive activity
causing elevation of mean arterial blood pressure,
indicative of its being a Ucn antagonist.

EXAMPLE XII

30   The peptide, (cyclo 29-32)[D-Tyr^{11}, Glu^{29}, Lys^{32}]-
Ucn(11-40), having the amino acid sequence:
H-D-Tyr-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
Gln-Ser-Gln-Arg-Glu-Glu-Ala-Glu-Lys-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in

Example I, with the cyclizing lactam bond being created as
described in Example I of U.S. Patent No. 5,064,939.
LSIMS shows a value of 3609.82 Da which agrees with the
calculated value of 3609.96 Da. *In vitro* testing for ACTH
5 secretion using anterior pituitary cell cultures as set
forth in Example VIII shows that the peptide is about 4
times as effective as the Standard Antagonist, i.e. 4.01
(2.32 - 7.05). The peptide has significant mammalian
vasoconstrictive activity causing elevation of mean
10 arterial blood pressure, indicative of its being a Ucn
antagonist. It is also iodinated to provide ^{125}I-D-Tyr^{11}
cyclic analog for use in screening assays and the like.

EXAMPLE XIIA

15   The peptide, (cyclo 29-32)[D-Tyr^{11}, Glu^{29}, D-Glu^{31},
Lys^{32}]-Ucn(11-40), having the amino acid sequence:
H-D-Tyr-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
Gln-Ser-Gln-Arg-Glu-Glu-Ala-D-Glu-Lys-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
20 Example I, with the cyclizing lactam bond being created as
described in Example I of U.S. Patent No. 5,064,939. The
peptide has significant mammalian vasoconstrictive
activity causing elevation of mean arterial blood
pressure, indicative of its being a Ucn antagonist. It is
25 also iodinated to provide ^{125}I-D-Tyr^{11} cyclic analog for use
in screening assays and the like.

EXAMPLE XIII

The peptide, (cyclo 29-32)[D-Phe^{11}, Glu^{29}, D-Glu^{31},
30 Lys^{32}]-Ucn(11-40), having the amino acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
Gln-Ser-Gln-Arg-Glu-Glu-Ala-D-Glu-Lys-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
Example I, with the cyclizing lactam bond being created as
35 described in Example I of U.S. Patent No. 5,064,939.

LSIMS shows a value of 3593.80 Da which agrees with the
calculated value of 3593.97. _In vitro_ testing for ACTH
secretion using anterior pituitary cell cultures as set
forth in Example VIII shows that the peptide is about 4.75
5 times as effective as the Standard Antagonist, i.e. 4.72
(2.19 - 10.00). The peptide also has significant
mammalian vasoconstrictive activity causing elevation of
mean arterial blood pressure, indicative of its being a
Ucn antagonist.

10   The synthesis is repeated twice to substitute D-Leu
and D-His for D-Phe. The peptides show similar
bioactivity as Ucn antagonists.

EXAMPLE XIIIA

15   The peptide, (cyclo 29-32)[Pro^{10},D-Phe^{11}, Glu^{29}, D-
Glu^{31}, Lys^{32}]-Ucn(10-40), having the amino acid sequence:
H-Pro-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-
Thr-Gln-Ser-Gln-Arg-Glu-Glu-Ala-D-Glu-Lys-Asn-Arg-Ile-Ile-
Phe-Asp-Ser-Val-NH_2 is synthesized in the manner described
20 in Example I, with the cyclizing lactam bond being created
as described in Example I of U.S. Patent No. 5,064,939.
LSIMS shows a value of 3690.91 Da which agrees with the
calculated value of 3691.02 Da. _In vitro_ testing for ACTH
secretion using anterior pituitary cell cultures as set
25 forth in Example VIII shows that the peptide is about 2.75
times as effective as the Standard Antagonist, i.e. 2.74
(1.02 - 8.02). The peptide also has significant mammalian
vasoconstrictive activity causing elevation of mean
arterial blood pressure, indicative of its being a Ucn
30 antagonist. This synthesis and testing show that the
inclusion of an additional L-isomer at the N-terminus does
not significantly alter its bioactivity as a Ucn
antagonist.

35   **EXAMPLE XIIIB**

The peptide, (cyclo 29-32)[D-Pro^{10},D-Phe^{11}, Glu^{29}, D-Glu^{31}, Lys^{32}]-Ucn(10-40), having the amino acid sequence:
H-D-Pro-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Glu-Ala-D-Glu-Lys-Asn-Arg-Ile-Ile-
5 Phe-Asp-Ser-Val-NH$_2$ is synthesized in the manner described in Example I, with the cyclizing lactam bond being created as described in Example I of U.S. Patent No. 5,064,939. LSIMS shows a value of 3691.00 Da which agrees with the calculated value of 3691.02 Da. In vitro testing for ACTH
10 secretion using anterior pituitary cell cultures as set forth in Example VIII shows that the peptide is about 5 times as effective as the Standard Antagonist, i.e. 4.99 (2.26 - 11.55). The peptide also has significant mammalian vasoconstrictive activity causing elevation of
15 mean arterial blood pressure, indicative of its being a Ucn antagonist. This synthesis and testing show that the inclusion of an additional D-isomer at the N-terminus does not significantly alter the bioactivity of a Ucn antagonist, as by comparison to Peptide XIII.
20

EXAMPLE XIV

The peptide, (cyclo 29-32)[D-Phe^{11}, Glu^{29}, D-Arg^{31}, Orn^{32}]-Ucn(11-40), having the amino acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
25 Gln-Ser-Gln-Arg-Glu-Glu-Ala-D-Arg-Orn-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH$_2$ is synthesized in the manner described in Example I, with the cyclizing lactam bond being created as described in Example I of U.S. Patent No. 5,064,939. The peptide has significant mammalian vasoconstrictive
30 activity causing elevation of mean arterial blood pressure, indicative of its being a Ucn antagonist.

The synthesis is repeated twice substituting imBzlD-His and D-2Nal for D-Arg and twice again to also add Ac-Thr at the N-terminus with these substitutions. All four
35 peptides show good bioactivity as Ucn antagonists.

EXAMPLE XV

The peptide, (cyclo 29-32)[D-Phe^{11}, Lys^{29}, Glu^{32}]-
Ucn(11-40), having the amino acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
5 Gln-Ser-Gln-Arg-Glu-Lys-Ala-Glu-Glu-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
Example I, with the cyclizing lactam bond being created as
described in Example I of U.S. Patent No. 5,064,939. The
peptide has significant mammalian vasoconstrictive
10 activity causing elevation of mean arterial blood
pressure, indicative of its being a Ucn antagonist.

EXAMPLE XVI

The peptide, (cyclo 29-32)[D-Phe^{11}, Lys^{29}, D-Glu^{31},
15 Glu^{32}]-Ucn(11-40), having the amino acid sequence:
H-D-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-
Gln-Ser-Gln-Arg-Glu-Lys-Ala-D-Glu-Glu-Asn-Arg-Ile-Ile-Phe-
Asp-Ser-Val-NH$_2$ is synthesized in the manner described in
Example I, with the cyclizing lactam bond being created as
20 described in Example I of U.S. Patent No. 5,064,939. The
peptide has significant mammalian vasoconstrictive
activity causing elevation of mean arterial blood
pressure, indicative of its being a Ucn antagonist.

25

EXAMPLE XVII

Additional Ucn antagonist peptides, as set forth as
follows, many of which are considered to be substantially
30 the same as Ucn(11-40), are synthesized in the manner
described in Example I:

[Val^{17}]-rUcn(11-40)   [C$^{\alpha}$MeLeu^{36}]-rUcn(11-40)
[Lys^{22}]-rUcn(8-40)   [D-Phe^{11}, Ile^{18}]-rUcn(11-40)
35 [Ac-Asp^{8},Lys^{22}]-Ucn(8-40) [Leu^{35}]-rUcn(11-40)

[Ile^{20}]-rUcn(10-40)   [Ac-Thr^{10},Ile^{20}]-Ucn(10-40)

[Glu^{38}]-rUcn(11-40)   [D-Phe^{11}, Lys^{27}]-rUcn(11-40)

[Ser^{10}]-rUcn(10-40)   [C$^{\alpha}$MeIle^{36}, Leu^{37}]-rUcn(11-40)

[D-Leu^{11}]-rUcn(11-40)   [Ala^{38},Ile^{40}]-rUcn(11-40)

5 [Gln^{29}]-rUcn(9-40)   [Ala^{19}, Thr^{39}]-rUcn(11-40)

[Asn^{32}]-rUcn(11-40)   [D-Phe^{11}, Lys^{22,27}]-rUcn(11-40)

[Ac-Leu^{9},Gln^{29}]-Ucn(9-40)   [Lys^{22}, Gln^{29}]-rUcn(11-40)

Each of the foregoing Ucn-like antagonist peptides has

10 significant mammalian vasoconstrictive activity causing

elevation of mean arterial blood pressure, indicative of

its being a Ucn antagonist.

The following group of Examples are directed to the

synthesis of CRF-BP blockers which increase the available

15 amount of endogenous CRF and Ucn by complexing with CRF-

BP.

EXAMPLE XVIII

A peptide, Ucn(5-32), having the amino acid sequence

(see SEQ ID NO:8):

20 Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-

Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln is

synthesized in the manner described in Example I.

A prospective peptide blocker is evaluated using a

competitive hCRF-BP ligand binding assay. Binding of [^{125}I-

25 DTyr^{0}]hCRF to soluble hCRF-BP is performed in phosphate-

buffered saline (PBS) containing 25 mM EDTA, 0.25% bovine

serum albumin, and .01% Triton X-100, using medium

enriched by recombinant CHO cells as a source of hCRF-BP.

Reactions are performed in a total volume of 400 μl

30 including 50,000 CPM[^{125}I-DTyr^{0}]hCRF. A constant amount of

radioactive hCRF and hCRF-BP and varying amounts of the

sample peptide are used to carry out competitive binding

assays. After an overnight incubation at room

temperature, precipitation is accomplished using rabbit

35 anti-hCRF-BP antiserum (1:9000 final dilution) and 200 μl

of sheep anti-rabbit IgG solution. After incubating with
the primary and secondary antisera for 30 minutes each, 1
ml of saline wash is added, and the test tubes are
centrifuged at 2000 x g for 20 minutes at 4°C.

5 Precipitates are counted in a gamma counter. Inhibitory
binding affinity constant (K_i) values are determined using
parameters calculated by the LIGAND computer program,
Munson et al., *Anal. Biochem.*, *107*, 220 (1980), and a
Vax/VMS computer system.

10   The CRF-BP blocker Ucn(5-32) has a K_i lower than that
of hCRF(9-33) and thus is a potentially superior blocking
agent for increasing the available amount of CRF and/or
Ucn.

15   **EXAMPLE XIX**

        A peptide, Ucn(8-32), having the amino acid sequence
(see SEQ ID NO:8):
        Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-
        Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln is synthesized
20 in the manner described in Example I. LDMS shows a value
of 3023.48 Da which agrees with the calculated value of
3023.65 Da. Testing shows that the peptide has a K_i lower
than that of hCRF(9-33) and thus is a potentially superior
blocking agent for increasing the available amount of CRF
25 and/or Ucn.

                          EXAMPLE XX

        A peptide, Ucn(3-27), having the amino acid sequence
(see SEQ ID NO:8):
30 Pro-Pro-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-
        Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg is synthesized
in the manner described in Example I. The peptide has a K_i
lower than that of hCRF(9-33) and thus is a potentially
superior blocking agent for increasing the available
35 amount of CRF and/or Ucn.

EXAMPLE XXI

A peptide, [Ile^{18}]-Ucn(6-29), having the amino acid
sequence (see SEQ ID NO:8):
Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Ile-Glu-
5 Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg is synthesized in
the manner described in Example I. The peptide has a K_i
lower than that of hCRF(9-33) and thus is a potentially
superior blocking agent for increasing the available
amount of CRF and/or Ucn.

10   Ucn profoundly stimulates the pituitary-
adrenalcortical axis and is considered to be useful to
stimulate the functions of this axis in some types of
patients with low endogenous glucocorticoid production.
For example, Ucn and its analogs should be useful in
15 restoring pituitary-adrenal function in patients having
received exogenous glucocorticoid therapy whose
pituitary-adrenalcortical functions remain suppressed.

Most other regulatory peptides have been found to
have effects upon the central nervous system and upon the
20 gastrointestinal tract. Because ACTH and sympathetic
nervous system activation secretion is the "sine qua non"
of mammal's response to stress, it was not surprising that
CRFs have significant effects on the brain as a mediator
of the body's stress response. Accordingly, Ucn and its
25 analogs are considered to also find application in
modifying the mood, learning, memory and behavior of
normal and mentally disordered individuals. Because Ucn
elevates the levels of ACTH, β-END, β-lipotropin, other
pro-opiomelanocortin gene products and corticosterone, its
30 administration can be used to induce the effects of the
foregoing POMC-derived peptides on the brain to thereby
influence memory, mood, pain appreciation, etc., and more
specifically, alertness, depression and/or anxiety, and
also their effects peripherally. For example, when
35 administered directly into the ventricles, CRFs increase

physical activity and improve learning performance in rats
and thus may function as a natural stimulant; because Ucn
similarly activates the CRF receptors, it will function
similarly.

5   Because CRF-R2 has been found to be abundantly
expressed in the heart, especially in association with
blood vessels, and because it is known that the addition
of CRF into the left atrium of an isolated perfused heart
induces a prolonged dilatory effect on coronary arteries,
10 transiently produces a positive inotropic effect and
stimulates the secretion of atrial natriuretic peptide, it
is now believed that Ucn is responsible, at least in part,
for regulating cardiac perfusion because of its
particularly high affinity for the CRF-R2s. It is also
15 expected that other vascular beds, such as the superior
mesenteric, will be dilated by Ucn and its analogs.
Because of these biological effects in the heart, Ucn and
agonists/antagonists thereof (as well as anti-Ucn
antibodies), can be effectively used to selectively
20 modulate cardiac perfusion.

        Moreover, because of the localization of CRF-R2 on
blood vessels, it is considered that Ucn-like agonist and
antagonist peptides of the invention are therapeutically
useful to modulate blood flow in many various vascular
25 beds, and particularly in desired tissues and organs. Ucn
and its agonist analogs are considered to be of use for
increasing blood flow to the gastrointestinal tract of
animals, particularly humans and other mammals, because
all CRF-related peptides have been shown to dilate the
30 mesenteric vascular bed. CRF and certain fragments have
been shown to modulate vascular permeability (Wei E.T. et
al., "Peripheral anti-inflammatory actions of
corticotropin-releasing factor", pp. 258-276,
Corticotropin-Releasing Factor (Ciba Foundation Symposium
35 172) John Wiley & Sons, 1993). Ucn and its fragments will

also reduce vascular leakage and have a salutary effect on
injury- or surgery-induced tissue swelling and
inflammation. Therefore, Ucn and its analogs and
fragments that are agonists can be administered
5 parenterally to decrease inflammation, swelling and oedema
and to reduce fluid loss following heat injury.

oCRF, r/hCRF, urotensin I and sauvagine have been
shown to inhibit gastric acid production, and Ucn and its
analogs are considered to also be effective in the
10 treatment of gastric ulcers by reducing gastric acid
production and/or inhibiting certain gastrointestinal
functions in a mammal. Ucn and its analogs will be
effective in increasing intestinal transit rate and useful
in the treatment of acute constipation.

15   A number of direct stimulatory effects of CRF on the
GI tract have earlier been described. For example, CRF
acts on the gut *in vitro* to depolarize myenteric neurons
in the small intestine. The results of *in vivo* studies
with intravenously administered CRF and CRF antagonists
20 have been consistent with the observed effect of CRF to
control gastric emptying and intestinal motility. The
Ucn-like peptides of the invention are considered useful
in treating intestinal and gastrointestinal disorders,
such as irritable bowel syndrome. CRF antagonists have
25 previously been used to therapeutically treat irritable
bowel syndrome, and antagonists based upon Ucn (which
would be selective for CRF-R2) are considered to be even
more useful. These antagonists may also be used to treat
spastic colon and Crohn's disease.

30   These Ucn-like peptides may also be used to evaluate
hypothalamic pituitary adrenal function in mammals with
suspected endocrine or central nervous system pathology by
suitable administration followed by monitoring bodily
functions. For example, administration may be used as a

diagnostic tool to evaluate Cushing's disease and
affective disorders, such as depressive illness.

        Ucn, an analog or a nontoxic salt thereof, combined
with a pharmaceutically or veterinarily acceptable carrier
5 to form a pharmaceutical composition, may be administered
to animals, including humans and other mammals, either
intravenously, subcutaneously, intramuscularly,
percutaneously, e.g. intranasally, intracerebrospinally or
orally. The isolated peptides should be at least about
10 90% pure and preferably should have a purity of at least
about 98%; however, lower purities are effective and may
well be used with mammals other than humans. This purity
means that the intended peptide constitutes the stated
weight % of all like peptides and peptide fragments
15 present. Administration to humans may be employed by a
physician to lower blood pressure or to stimulate
endogenous gluco-corticoid production. The required
dosage will vary with the particular condition being
treated, with the severity of the condition and with the
20 duration of desired treatment. Ucn or Ucn analogs can
also be administered, e.g., icv, to cause an increase of
Ucn in the brain and thereby cause (a) improvement in
short to medium term memory in a subject afflicted with
Alzheimer's disease; (b) relief from chronic fatigue
25 syndrome; (c) suppression of appetite; (d) stimulation of
the respiratory system, (e) improvement in learning
performance; (f) improvement in memory; (g) improvement in
alertness; (h) reduction of depression and/or (i)
lessening of anxiety. Particular effectiveness is shown
30 in appetite suppression.

        Such peptides are often administered in the form of
pharmaceutically or veterinarily acceptable nontoxic
salts, such as acid addition salts or metal complexes,
e.g., with zinc, iron, calcium, barium, magnesium,
35 aluminum or the like. Illustrative of such nontoxic salts

are hydrochloride, hydrobromide, sulphate, phosphate, tannate, oxalate, fumarate, gluconate, alginate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be

5 administered in tablet form, the tablet may contain a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring may

10 be used, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

It may also be desirable to deliver Ucn or analogs thereof over prolonged periods of time, for example, for

15 periods of one week to one year from a single administration, and slow release, depot or implant dosage forms may be utilized as well known in this art. For example, a dosage form may contain a pharmaceutically acceptable non-toxic salt of the compound which has a low

20 degree of solubility in body fluids, for example, an acid addition salt with a polybasic acid; a salt with a polyvalent metal cation; or combination of the two salts. A relatively insoluble salt may also be formulated in a gel, for example, an aluminum stearate gel. A suitable,

25 slow-release depot formulation for injection may also contain Ucn or an analog or a salt thereof dispersed or encapsulated in a slow degrading, non-toxic or non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer, for example, as described

30 in U.S. Pat. No. 3,773,919.

Therapeutically effective amounts of the peptides should be administered under the guidance of a physician, and pharmaceutical compositions will usually contain the peptide in conjunction with a conventional,

35 pharmaceutically or veterinarily-acceptable carrier. A

therapeutically effective amount is considered to be a predetermined amount calculated to achieve the desired effect, e.g. to increase or decrease the amount of ACTH, in a patient. The required dosage will vary with the

5 particular treatment and with the duration of desired treatment; however, it is anticipated that dosages between about 10 micrograms and about 1 milligram per kilogram of body weight per day will be used for therapeutic treatment. It may be particularly advantageous to

10 administer such compounds in depot or long-lasting form as earlier described. A therapeutically effective amount is typically an amount of a Ucn or an analog thereof that, when administered peripherally in a physiologically acceptable composition, is sufficient to achieve a plasma

15 concentration thereof from about 0.1 μg/ml to about 100 μg/ml, preferably from about 1 μg/ml to about 50 μg/ml, more preferably at least about 2 μg/ml and usually 5 to 10 μg/ml. Antibodies or antisense polynucleotides are also administered in proportionately appropriate amounts in

20 accordance with known practices in this art. The level of ACTH present in a patient, particularly in the plasma, can be readily determined by routine clinical analysis. Changes in ACTH levels can be monitored during a treatment regimen to determine the effectiveness of the administered

25 Ucn-like peptide over time. In some instances, treatment of subjects with these peptides can be carried out in lieu of the administration of ACTH or corticosteroids, in such instances a dosage as low as about 10 ng/Kg of body weight may be employed.

30 Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art

35 may be made without departing from the scope of the

invention which is set forth in the claims appended
hereto. Although the claims variously define the
invention in terms of a peptide sequence, it should be
understood that such is intended to include nontoxic salts
5 thereof which are well known to be the full equivalent
thereof and which are most frequently administered.
Instead of the simple amide at the C-terminus, a lower
alkyl-substituted amide, e.g. methylamide, ethylamide,
etc, may be incorporated or the C-terminus may be
10 otherwise blocked as well known in the peptide art.
Polypeptides having an amino acid residue sequence
substantially identical to the sequence of Ucn
specifically shown herein, in which one or more residues
have been conservatively substituted with a functionally
15 similar residue, are considered to be equivalents of Ucn
so long as they mimic a biological function of CRF. Such
peptides and salts thereof are considered as being within
the scope of the claimed invention.

The disclosures of all patents and publications set
20 forth hereinbefore, as well as of the two priority
applications, are expressly incorporated herein by
reference. As used herein, all temperatures are °C., and
all ratios and percentages of liquid materials are by
volume.

25

Sequence Listing Summary

SEQ ID NO:1, when the C-terminus is amidated, is the amino acid sequence of ovine CRF.

SEQ ID NO:2, when pGlu is at the N-terminus and the
5 C-terminus is amidated, is the amino acid sequence of frog sauvagine.

SEQ ID NO:3, when the C-terminus is amidated, is the amino acid sequence of rat/human CRF.

SEQ ID NO:4, when the C-terminus is amidated, is the
10 amino acid sequence of suckerfish urotensin.

SEQ ID NO:5, when the C-terminus is amidated, is the amino acid sequence of carp urotensin.

SEQ ID NO:6, when the C-terminus is amidated, is the amino acid sequence of flathead sole (Maggy).

15   SEQ ID NO:7, when the C-terminus is amidated, is the amino acid sequence of fish CRF.

SEQ ID NO:8, when the C-terminus is amidated, is the amino acid sequence of rat-derived urocortin(Ucn).

SEQ ID NO:9 is the nucleic acid sequence from which
20 SEQ ID NO:8 was deduced.

SEQ ID NO:10 is the amino acid sequence of the rat-derived CRF receptor referred to as "rCRF-R1".

SEQ ID NO:11 is the amino acid sequence of a mouse-derived CRF receptor referred to as "mCRF-R2β".

25   SEQ ID NO:12 is the amino acid sequence of a rat-derived CRF receptor referred to as "rCRF-R2α".

SEQ ID NO:13 is the amino acid sequence of a rat-derived CRF receptor referred to as "rCRF-R2β".

SEQ ID NO:14 is the amino acid sequence of a 40-
30 residue peptide defining certain analogs of Ucn.

SEQ ID NO:15 is the amino acid sequence of the precursor plus the mature human Ucn peptide.

SEQ ID NO:16 is the nucleic acid sequence from which SEQ ID NO:15 was deduced.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: UROCORTIN PEPTIDES

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(v) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Floppy disk
    (B) COMPUTER: IBM PC compatible
    (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
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(vi) PRIOR APPLICATION DATA:
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    (B) FILING DATE: 11-AUG-1995

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    (C) REFERENCE/DOCKET NUMBER: 57611-PC

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 41 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ser Gln Glu Pro Pro Ile Ser Leu Asp Leu Thr Phe His Leu Leu Arg
1               5                   10                  15

Glu Val Leu Glu Met Thr Lys Ala Asp Gln Leu Ala Gln Gln Ala His
            20                  25                  30

Ser Asn Arg Lys Leu Leu Asp Ile Ala
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH: 40 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Gly Pro Pro Ile Ser Ile Asp Leu Ser Leu Glu Leu Leu Arg Lys
 1   5   10   15

Met Ile Glu Ile Glu Lys Gln Glu Lys Glu Lys Gln Gln Ala Ala Asn
            20   25   30

Asn Arg Leu Leu Leu Asp Thr Ile
        35   40

     (2) INFORMATION FOR SEQ ID NO:3:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 41 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Glu Glu Pro Pro Ile Ser Leu Asp Leu Thr Phe His Leu Leu Arg
 1   5   10   15

Glu Val Leu Glu Met Ala Arg Ala Glu Gln Leu Ala Gln Gln Ala His
            20   25   30

Ser Asn Arg Lys Leu Met Glu Ile Ile
        35   40

     (2) INFORMATION FOR SEQ ID NO:4:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 41 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asn Asp Asp Pro Pro Ile Ser Ile Asp Leu Thr Phe His Leu Leu Arg
 1   5   10   15

Asn Met Ile Glu Met Ala Arg Ile Glu Asn Glu Arg Glu Gln Ala Gly
            20   25   30

Leu Asn Arg Lys Tyr Leu Asp Glu Val
        35   40

     (2) INFORMATION FOR SEQ ID NO:5:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 41 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asn Asp Asp Pro Pro Ile Ser Ile Asp Leu Thr Phe His Leu Leu Arg
1   5   10   15

Asn Met Ile Glu Met Ala Arg Asn Glu Asn Gln Arg Glu Gln Ala Gly
            20   25   30

Leu Asn Arg Lys Tyr Leu Asp Glu Val
        35   40

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 41 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Glu Glu Pro Pro Met Ser Ile Asp Leu Thr Phe His Met Leu Arg
1   5   10   15

Asn Met Ile His Arg Ala Lys Met Glu Gly Glu Arg Glu Gln Ala Leu
            20   25   30

Ile Asn Arg Asn Leu Leu Asp Glu Val
        35   40

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 41 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Glu Glu Pro Pro Ile Ser Leu Asp Leu Thr Phe His Leu Leu Arg
1   5   10   15

Glu Val Leu Glu Met Ala Arg Ala Glu Gln Leu Ala Gln Gln Ala His
            20   25   30

Ser Asn Arg Lys Met Met Glu Ile Phe
        35   40

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 40 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

-72-

```
Asp Asp Pro Pro Leu Ser Ile Asp Leu Thr Phe His Leu Leu Arg Thr
1               5                   10                  15

Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln
            20                  25                  30

Asn Arg Ile Ile Phe Asp Ser Val
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 129 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: cDNA to mRNA

      (iii) HYPOTHETICAL: NO

       (iv) ANTI-SENSE: NO

       (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Rat Brain

      (vii) IMMEDIATE SOURCE:
            (B) CLONE: CK21

       (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 1..120

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GAC GAC CCG CCG TTG TCC ATC GAC CTC ACC TTC CAC CTG CTG CGG ACC        48
Asp Asp Pro Pro Leu Ser Ile Asp Leu Thr Phe His Leu Leu Arg Thr
1               5                   10                  15

CTG CTA GAG CTA GCT CGG ACA CAG AGC CAG CGC GAG CGC GCA GAG CAG        96
Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala Glu Gln
            20                  25                  30

AAC CGC ATC ATA TTC GAT TCG GTG GGCAAGTGA                            129
Asn Arg Ile Ile Phe Asp Ser Val
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 415 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

       (ix) FEATURE:
            (A) NAME/KEY: Protein
            (B) LOCATION: 1..415
            (D) OTHER INFORMATION: /note= "Human Pituitary
CRF-Receptor-R1"

-73-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Gly Arg Arg Pro Gln Leu Arg Leu Val Lys Ala Leu Leu Leu Leu
 1               5                  10              15

Gly Leu Asn Pro Val Ser Thr Ser Leu Gln Asp Gln Arg Cys Glu Asn
            20              25              30

Leu Ser Leu Thr Ser Asn Val Ser Gly Leu Gln Cys Asn Ala Ser Val
        35              40              45

Asp Leu Ile Gly Thr Cys Trp Pro Arg Ser Pro Ala Gly Gln Leu Val
        50              55              60

Val Arg Pro Cys Pro Ala Phe Phe Tyr Gly Val Arg Tyr Asn Thr Thr
65              70              75              80

Asn Asn Gly Tyr Arg Glu Cys Leu Ala Asn Gly Ser Trp Ala Ala Arg
            85              90              95

Val Asn Tyr Ser Glu Cys Gln Glu Ile Leu Asn Glu Glu Lys Lys Ser
        100             105             110

Lys Val His Tyr His Val Ala Val Ile Ile Asn Tyr Leu Gly His Cys
        115             120             125

Ile Ser Leu Val Ala Leu Leu Val Ala Phe Val Leu Phe Leu Arg Leu
    130             135             140

Arg Ser Ile Arg Cys Leu Arg Asn Ile Ile His Trp Asn Leu Ile Ser
145             150             155             160

Ala Phe Ile Leu Arg Asn Ala Thr Trp Phe Val Val Gln Leu Thr Val
            165             170             175

Ser Pro Glu Val His Gln Ser Asn Val Ala Trp Cys Arg Leu Val Thr
            180             185             190

Ala Ala Tyr Asn Tyr Phe His Val Thr Asn Phe Phe Trp Met Phe Gly
        195             200             205

Glu Gly Cys Tyr Leu His Thr Ala Ile Val Leu Thr Tyr Ser Thr Asp
    210             215             220

Arg Leu Arg Lys Trp Met Phe Val Cys Ile Gly Trp Gly Val Pro Phe
225             230             235             240
```

```
Pro Ile Ile Val Ala Trp Ala Ile Gly Lys Leu His Tyr Asp Asn Glu
             245                 250                 255

Lys Cys Trp Phe Gly Lys Arg Pro Gly Val Tyr Thr Asp Tyr Ile Tyr
             260                 265                 270

Gln Gly Pro Met Ile Leu Val Leu Leu Ile Asn Phe Ile Phe Leu Phe
             275                 280                 285

Asn Ile Val Arg Ile Leu Met Thr Lys Leu Arg Ala Ser Thr Thr Ser
     290                 295                 300

Glu Thr Ile Gln Tyr Arg Lys Ala Val Lys Ala Thr Leu Val Leu Leu
305                 310                 315                 320

Pro Leu Leu Gly Ile Thr Tyr Met Leu Phe Phe Val Asn Pro Gly Glu
             325                 330                 335

Asp Glu Val Ser Arg Val Val Phe Ile Tyr Phe Asn Ser Phe Leu Glu
             340                 345                 350

Ser Phe Gln Gly Phe Phe Val Ser Val Phe Tyr Cys Phe Leu Asn Ser
             355                 360                 365

Glu Val Arg Ser Ala Ile Arg Lys Arg Trp Arg Arg Trp Gln Asp Lys
     370                 375                 380

His Ser Ile Arg Ala Arg Val Ala Arg Ala Met Ser Ile Pro Thr Ser
385                 390                 395                 400

Pro Thr Arg Val Ser Phe His Ser Ile Lys Gln Ser Thr Ala Val
             405                 410                 415
```

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 431 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..431
        (D) OTHER INFORMATION: /note= "Product-mouse heart derived
CRF-R2 Long Form"

    (x) PUBLICATION INFORMATION:
        (A) AUTHORS: Perrin, Marilyn
                    Donaldson, Cynthia
                    Chen, Ruoping
                    Blount, Amy
                    Berggren, Travis
                    Bilezikjian, Louise
                    Sawchenko, Paul
                    Vale, Wylie

        (B) TITLE: Identification of a second
                  corticotropin-releasing factor receptor gene and
                  characterization of a cDNA expressed in heart
        (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
        (D) VOLUME: 92

    (F) PAGES: 2969-2973
    (G) DATE: March-1995

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Gly Thr Pro Gly Ser Leu Pro Ser Ala Gln Leu Leu Leu Cys Leu
1   5   10   15

Phe Ser Leu Leu Pro Val Leu Gln Val Ala Gln Pro Gly Gln Ala Pro
            20   25   30

Gln Asp Gln Pro Leu Trp Thr Leu Leu Glu Gln Tyr Cys His Arg Thr
        35   40   45

Thr Ile Gly Asn Phe Ser Gly Pro Tyr Thr Tyr Cys Asn Thr Thr Leu
        50   55   60

Asp Gln Ile Gly Thr Cys Trp Pro Gln Ser Ala Pro Gly Ala Leu Val
65   70   75   80

Glu Arg Pro Cys Pro Glu Tyr Phe Asn Gly Ile Lys Tyr Asn Thr Thr
                85   90   95

Arg Asn Ala Tyr Arg Glu Cys Leu Glu Asn Gly Thr Trp Ala Ser Arg
            100   105   110

Val Asn Tyr Ser His Cys Glu Pro Ile Leu Asp Asp Lys Gln Arg Lys
        115   120   125

Tyr Asp Leu His Tyr Arg Ile Ala Leu Ile Val Asn Tyr Leu Gly His
    130   135   140

Cys Val Ser Val Val Ala Leu Val Ala Ala Phe Leu Leu Phe Leu Val
145   150   155   160

Leu Arg Ser Ile Arg Cys Leu Arg Asn Val Ile His Trp Asn Leu Ile
            165   170   175

Thr Thr Phe Ile Leu Arg Asn Ile Ala Trp Phe Leu Leu Gln Leu Ile
            180   185   190

Asp His Glu Val His Glu Gly Asn Glu Val Trp Cys Arg Cys Ile Thr
        195   200   205

Thr Ile Phe Asn Tyr Phe Val Val Thr Asn Phe Phe Trp Met Phe Val
    210   215   220

Glu Gly Cys Tyr Leu His Thr Ala Ile Val Met Thr Tyr Ser Thr Glu
225   230   235   240

His Leu Arg Lys Trp Leu Phe Leu Phe Ile Gly Trp Cys Ile Pro Cys
            245   250   255

Pro Ile Ile Ile Ala Trp Ala Val Gly Lys Leu Tyr Tyr Glu Asn Glu
            260   265   270

Gln Cys Trp Phe Gly Lys Glu Ala Gly Asp Leu Val Asp Tyr Ile Tyr
        275   280   285

Gln Gly Pro Val Met Leu Val Leu Leu Ile Asn Phe Val Phe Leu Phe
    290   295   300

```
Asn Ile Val Arg Ile Leu Met Thr Lys Leu Arg Ala Ser Thr Thr Ser
305                 310             315             320

Glu Thr Ile Gln Tyr Arg Lys Ala Val Lys Ala Thr Leu Val Leu Leu
                325             330             335

Pro Leu Leu Gly Ile Thr Tyr Met Leu Phe Phe Val Asn Pro Gly Glu
            340             345             350

Asp Asp Leu Ser Gln Ile Val Phe Ile Tyr Phe Asn Ser Phe Leu Gln
            355             360             365

Ser Phe Gln Gly Phe Phe Val Ser Val Phe Tyr Cys Phe Phe Asn Gly
    370             375             380

Glu Val Arg Ala Ala Leu Arg Lys Arg Trp His Arg Trp Gln Asp His
385             390             395             400

His Ala Leu Arg Val Pro Val Ala Arg Ala Met Ser Ile Pro Thr Ser
            405             410             415

Pro Thr Arg Ile Ser Phe His Ser Ile Lys Gln Thr Ala Ala Val
            420             425             430
```

(2) INFORMATION FOR SEQ ID NO:12:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 411 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (ix) FEATURE:
            (A) NAME/KEY: Protein
            (B) LOCATION: 1..411
            (D) OTHER INFORMATION: /note= "Rat CRF-R2 Short Form"

        (x) PUBLICATION INFORMATION:
            (A) AUTHORS: Lovenberg, Timothy W
                         Liaw, Chen W
                         Grigoriadis, Dimitri E
                         Clevenger, William
                         Chalmers, Derek T
                         DeSouza, Errol B
                         Oltersdorf, Tilman
            (B) TITLE: Cloning and characterization of a
                functionally distinct corticotropin-releasing
                factor receptor subtype from rat brain
            (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
            (D) VOLUME: 92
            (F) PAGES: 836-840
            (G) DATE: January-1995

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Asp Ala Ala Leu Leu Leu Ser Leu Leu Glu Ala Asn Cys Ser Leu
1               5               10              15

Ala Leu Ala Glu Glu Leu Leu Leu Asp Gly Trp Gly Glu Pro Pro Asp
            20              25              30
```

```
Pro Glu Gly Pro Tyr Ser Tyr Cys Asn Thr Thr Leu Asp Gln Ile Gly
        35              40              45

Thr Cys Trp Pro Gln Ser Ala Pro Gly Ala Leu Val Glu Arg Pro Cys
    50              55              60

Pro Glu Tyr Phe Asn Gly Ile Lys Tyr Asn Thr Thr Arg Asn Ala Tyr
65              70              75              80

Arg Glu Cys Leu Glu Asn Gly Thr Trp Ala Ser Arg Ile Asn Tyr Ser
                85              90              95

His Cys Glu Pro Ile Leu Asp Asp Lys Gln Arg Lys Tyr Asp Leu His
            100             105             110

Tyr Arg Ile Ala Leu Ile Ile Asn Tyr Leu Gly His Cys Val Ser Val
        115             120             125

Val Ala Leu Val Ala Ala Phe Leu Leu Phe Leu Val Leu Arg Ser Ile
    130             135             140

Arg Cys Leu Arg Asn Val Ile His Trp Asn Leu Ile Thr Thr Phe Ile
145             150             155             160

Leu Arg Asn Ile Thr Trp Phe Leu Leu Gln Leu Ile Asp His Glu Val
                165             170             175

His Glu Gly Asn Glu Val Trp Cys Arg Cys Val Thr Thr Ile Phe Asn
            180             185             190

Tyr Phe Val Val Thr Asn Phe Phe Trp Met Phe Val Glu Gly Cys Tyr
        195             200             205

Leu His Thr Ala Ile Val Met Thr Tyr Ser Thr Glu His Leu Arg Lys
    210             215             220

Trp Leu Phe Leu Phe Ile Gly Trp Cys Ile Pro Cys Pro Ile Ile Val
225             230             235             240

Ala Trp Ala Val Gly Lys Leu Tyr Tyr Glu Asn Glu Gln Cys Trp Phe
                245             250             255

Gly Lys Glu Pro Gly Asp Leu Val Asp Tyr Ile Tyr Gln Gly Pro Ile
            260             265             270

Ile Leu Val Leu Leu Ile Asn Phe Val Phe Leu Phe Asn Ile Val Arg
        275             280             285

Ile Leu Met Thr Lys Leu Arg Ala Ser Thr Thr Ser Glu Thr Ile Gln
    290             295             300

Tyr Arg Lys Ala Val Lys Ala Thr Leu Val Leu Leu Pro Leu Leu Gly
305             310             315             320

Ile Thr Tyr Met Leu Phe Phe Val Asn Pro Gly Glu Asp Asp Leu Ser
            325             330             335

Gln Ile Val Phe Ile Tyr Phe Asn Ser Phe Leu Gln Ser Phe Gln Gly
        340             345             350

Phe Phe Val Ser Val Phe Tyr Cys Phe Phe Asn Gly Glu Val Arg Ser
    355             360             365
```

-78-

```
Ala Leu Arg Lys Arg Trp His Arg Trp Gln Asp His His Ala Leu Arg
370              375             380

Val Pro Val Ala Arg Ala Met Ser Ile Pro Thr Ser Pro Thr Arg Ile
385              390             395             400

Ser Phe His Ser Ile Lys Gln Thr Ala Ala Val
                 405             410
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 431 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..431
        (D) OTHER INFORMATION: /note= "Rat CRF-R2 Long Form"

    (x) PUBLICATION INFORMATION:
        (A) AUTHORS: Lovenberg, Timothy W
                Liaw, Chen W
                Grigoriadis, Dimitri E
                Clevenger, William
                Chalmers, Derek T
                DeSouza, Errol B
                Oltersdorf, Tilman
        (B) TITLE: Cloning and characterization of a
            functionally distinct corticotropin-releasing
            factor receptor subtype from rat brain
        (C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
        (D) VOLUME: 92
        (F) PAGES: 836-840
        (G) DATE: January-1995

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Gly His Pro Gly Ser Leu Pro Ser Ala Gln Leu Leu Leu Cys Leu
1               5               10              15

Tyr Ser Leu Leu Pro Leu Leu Gln Val Ala Gln Pro Gly Arg Pro Leu
            20              25              30

Gln Asp Gln Pro Leu Trp Thr Leu Leu Glu Gln Tyr Cys His Arg Thr
        35              40              45

Thr Thr Arg Asn Phe Ser Gly Pro Tyr Ser Tyr Cys Asn Thr Thr Leu
    50              55              60

Asp Gln Ile Gly Thr Cys Trp Pro Gln Ser Ala Pro Gly Ala Leu Val
65              70              75              80

Glu Arg Pro Cys Pro Glu Tyr Phe Asn Gly Ile Lys Tyr Asn Thr Thr
            85              90              95

Arg Asn Ala Tyr Arg Glu Cys Leu Glu Asn Gly Thr Trp Ala Ser Arg
            100             105             110
```

```
Ile Asn Tyr Ser His Cys Glu Pro Ile Leu Asp Asp Lys Gln Arg Lys
        115             120             125

Tyr Asp Leu His Tyr Arg Ile Ala Leu Ile Ile Asn Tyr Leu Gly His
    130             135             140

Cys Val Ser Val Val Ala Leu Val Ala Ala Phe Leu Leu Phe Leu Val
145             150             155             160

Leu Arg Ser Ile Arg Cys Leu Arg Asn Val Ile His Trp Asn Leu Ile
            165             170             175

Thr Thr Phe Ile Leu Arg Asn Ile Thr Trp Phe Leu Leu Gln Leu Ile
        180             185             190

Asp His Glu Val His Glu Gly Asn Glu Val Trp Cys Arg Cys Val Thr
        195             200             205

Thr Ile Phe Asn Tyr Phe Val Val Thr Asn Phe Phe Trp Met Phe Val
    210             215             220

Glu Gly Cys Tyr Leu His Thr Ala Ile Val Met Thr Tyr Ser Thr Glu
225             230             235             240

His Leu Arg Lys Trp Leu Phe Leu Phe Ile Gly Trp Cys Ile Pro Cys
            245             250             255

Pro Ile Ile Val Ala Trp Ala Val Gly Lys Leu Tyr Tyr Glu Asn Glu
            260             265             270

Gln Cys Trp Phe Gly Lys Glu Pro Gly Asp Leu Val Asp Tyr Ile Tyr
        275             280             285

Gln Gly Pro Ile Ile Leu Val Leu Leu Ile Asn Phe Val Phe Leu Phe
    290             295             300

Asn Ile Val Arg Ile Leu Met Thr Lys Leu Arg Ala Ser Thr Thr Ser
305             310             315             320

Glu Thr Ile Gln Tyr Arg Lys Ala Val Lys Ala Thr Leu Val Leu Leu
            325             330             335

Pro Leu Leu Gly Ile Thr Tyr Met Leu Phe Phe Val Asn Pro Gly Glu
            340             345             350

Asp Asp Leu Ser Gln Ile Val Phe Ile Tyr Phe Asn Ser Phe Leu Gln
        355             360             365

Ser Phe Gln Gly Phe Phe Val Ser Val Phe Tyr Cys Phe Phe Asn Gly
    370             375             380

Glu Val Arg Ser Ala Leu Arg Lys Arg Trp His Arg Trp Gln Asp His
385             390             395             400

His Ala Leu Arg Val Pro Val Ala Arg Ala Met Ser Ile Pro Thr Ser
            405             410             415

Pro Thr Arg Ile Ser Phe His Ser Ile Lys Gln Thr Ala Ala Val
        420             425             430
```

(2) INFORMATION FOR SEQ ID NO:14:

   (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 40 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Xaa Xaa Pro Xaa Xaa Ser Xaa Asp Leu Xaa Xaa Xaa Xaa Leu Arg Xaa
1   5   10   15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa
            20   25   30

Asn Arg Xaa Xaa Xaa Xaa Xaa Xaa
        35   40

(2) INFORMATION FOR SEQ ID NO: 15:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 125 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Arg Gln Ala Gly Arg Ala Ala Leu Leu Ala Ala Leu Leu Leu Leu
1   5   10   15

Val Gln Leu Cys Pro Gly Ser Ser Gln Arg Ser Pro Glu Ala Ala Gly
            20   25   30

Val Gln Asp Pro Ser Leu Arg Trp Ser Pro Gly Ala Arg Asn Gln Gly
        35   40   45

Gly Gly Ala Arg Ala Leu Leu Leu Leu Leu Ala Glu Arg Phe Pro Arg
    50   55   60

Arg Ala Gly Pro Gly Arg Leu Gly Leu Gly Thr Ala Gly Glu Arg Pro
65   70   75   80

Arg Arg Asp Asn Pro Ser Leu Ser Ile Asp Leu Thr Phe His Leu Leu
            85   90   95

Arg Thr Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala
            100   105   110

Glu Gln Asn Arg Ile Ile Phe Asp Ser Val Gly Lys   *
        115   120   125

(2) INFORMATION FOR SEQ ID NO: 16:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 375 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Human Genomic Placental Library

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION:1..375

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
ATG AGG CAG GCG GGA CGC GCA GCG CTG CTG GCC GCG CTG CTG CTC CTG    48
Met Arg Gln Ala Gly Arg Ala Ala Leu Leu Ala Ala Leu Leu Leu Leu
 1               5              10              15

GTA CAG CTG TGC CCT GGG AGC AGC CAG AGG AGC CCC GAG GCG GCC GGG    96
Val Gln Leu Cys Pro Gly Ser Ser Gln Arg Ser Pro Glu Ala Ala Gly
            20              25              30

GTC CAG GAC CCG AGT CTG CGC TGG AGC CCC GGG GCA CGG AAC CAG GGT   144
Val Gln Asp Pro Ser Leu Arg Trp Ser Pro Gly Ala Arg Asn Gln Gly
            35              40              45

GGC GGG GCC CGC GCG CTC CTC TTG CTG CTG GCG GAG CGC TTC CCG CGC   192
Gly Gly Ala Arg Ala Leu Leu Leu Leu Leu Ala Glu Arg Phe Pro Arg
        50              55              60

CGC GCG GGG CCC GGC CGA TTG GGA CTC GGG ACG GCA GGC GAG CGG CCG   240
Arg Ala Gly Pro Gly Arg Leu Gly Leu Gly Thr Ala Gly Glu Arg Pro
 65              70              75              80

CGG CGG GAC AAC CCT TCT CTG TCC ATT GAC CTC ACC TTT CAC CTG CTG   288
Arg Arg Asp Asn Pro Ser Leu Ser Ile Asp Leu Thr Phe His Leu Leu
                85              90              95

CGG ACC CTG CTG GAG CTG GCG CGG ACG CAG AGC CAG CGG GAG CGC GCC   336
Arg Thr Leu Leu Glu Leu Ala Arg Thr Gln Ser Gln Arg Glu Arg Ala
            100             105             110

GAG CAG AAC CGC ATC ATA TTC GAC TCG GTG GGC AAG TGA                375
Glu Gln Asn Arg Ile Ile Phe Asp Ser Val Gly Lys  *
            115             120             125
```

WHAT IS CLAIMED IS:

1. An isolated peptide which is characterized by binding to CRF receptor 2 (CRF-R2),

promoting production of ACTH,

exhibiting higher affinity for the long form of CRF-R2 than does rat/human CRF (r/hCRF), and

having less than about 80% homology with r/hCRF (SEQ ID NO:3), ovine CRF (SEQ ID NO:1) or carp Urotensin (SEQ ID NO:5).

2. A peptide according to Claim 1 comprising substantially the same amino acid sequence as residues 83-122 of SEQ ID NO: 15 or a biologically functional fragment thereof.

3. A peptide according to Claim 1 comprising residues 83-122 of amino acid sequence SEQ ID NO:15 or a biologically functional fragment thereof.

4. A peptide according to Claim 3 which is an N-terminally shortened functional fragment of the amino acid sequence of SEQ ID NO:15 wherein the C-terminus of said fragment is amidated.

5. A peptide according to Claim 4 wherein the N-terminus is acylated.

6. Antibodies which bind to a peptide according to Claim 3 or to a fragment thereof at least about 5 residues in length.

7. Antibodies according to Claim 6 which bind to and biologically inactivate said peptide.

8.   A peptide according to Claim 1 having at least
66% homology with residues 83-122 of SEQ ID NO:15 and
wherein all said substitutions for residues in SEQ ID
NO:15 are conservative substitutions.

9.   A peptide according to Claim 1 comprising
substantially the same sequence as the following amino
acid sequence:
Y-R_1-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-
Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-
Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val-NH_2 (residues
3-40 of SEQ ID NO:15), wherein Y is an acyl group having 7
or less carbon atoms or hydrogen; and R_1 is Asn or desR_1.

10.   A peptide according to Claim 1 comprising the
following amino acid sequence:
Y-Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-D-Phe-His-Leu-
Leu-Arg-Thr-Leu-Leu-R_{19}-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-
Glu-R_{29}-Ala-Glu-R_{32}-Asn-Arg-Ile-R_{36}-Phe-R_{38}-Ser-Val-NH_2,
wherein Y is an acyl group having 7 or less carbon atoms
or hydrogen; R_{19} is Glu or Ala; R_{29} is Arg, Glu, Lys or Orn;
R_{32} is Gln, Lys, Orn or Glu; R_{36} is Ile, C^{α}MeIle or C^{α}MeLeu;
R_{38} is Asp or Ala; provided that when R_{29} is Glu, R_{32} is
either Lys or Orn and the side chains thereof are linked
by an amide bond and that when R_{32} is Glu, R_{29} is either Lys
or Orn and the side chains thereof are linked by an amide
bond; and provided further that D-Phe in the 12-position
can be D-Leu or another D-amino acid; that Glu in the 31-
position can be D-Glu or another D-amino acid; and that
the N-terminus can be shortened by 1 or 2 residues.

11.   A peptide according to Claim 1 having the
following amino acid sequence (SEQ ID NO: 14):
Y-Xaa_1-Xaa_2-Pro-Xaa_4-Xaa_5-Ser-Xaa_7-Asp-Leu-Xaa_{10}-Xaa_{11}-Xaa_{12}-
Xaa_{13}-Leu-Arg-Xaa_{16}-Xaa_{17}-Xaa_{18}-Xaa_{19}-Xaa_{20}-Xaa_{21}-Xaa_{22}-Xaa_{23}-

$Xaa_{24}-Xaa_{25}-Xaa_{26}-Xaa_{27}-Xaa_{28}-Xaa_{29}-Ala-Xaa_{31}-Xaa_{32}-Asn-Arg-Xaa_{35}-$
$Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-NH_2$, wherein Y is an acyl group
having 7 or less carbon atoms or hydrogen; Xaa_1 is Asp, Glu
or Gln; Xaa_2 is Asn, Asp, Glu or Gly; Xaa_4 is Ser or Pro;
Xaa_5 is Leu, Ile or Met; Xaa_7 is Ile or Leu; Xaa_{10} is Thr or
Ser; Xaa_{11} is Phe or Leu; Xaa_{12} is His or Glu; Xaa_{13} is Leu
or Met; Xaa_{16} is Thr, Asn, Glu, or Lys; Xaa_{17} is Leu, Met or
Val; Xaa_{18} is Leu or Ile; Xaa_{19} is Glu or His; Xaa_{20} is Leu,
Met, Ile or Arg; Xaa_{21} is Ala, Glu or Thr; Xaa_{22} is Arg or
Lys; Xaa_{23} is any natural amino acid other than Cys; Xaa_{24}
is Gln, Glu or Asp; Xaa_{25} is any natural amino acid other
than Cys; Xaa_{26} is Gln, Leu or Glu; Xaa_{27} is Arg, Ala or
Lys; Xaa_{28} is Glu or Gln; Xaa_{29} is Arg or Gln; Xaa_{31} is any
natural amino acid other than Cys; Xaa_{32} is any natural
amino acid other than Cys; Xaa_{35} is Ile, Lys, Leu or Asn;
Xaa_{36} is Ile, Tyr, Met or Leu; Xaa_{37} is Phe, Leu or Met;
Xaa_{38} is Asp or Glu; Xaa_{39} is Ser, Ile, Glu or Thr; and Xaa_{40}
is Val, Ile, Phe or Ala; provided that there are no more
than 3 residues different from hUcn and that the N-
terminus can be shortened by 1 or 2 residues.

12.   Isolated DNA which encodes a peptide according
to Claim 1.

13.   Isolated DNA according to Claim 12 comprising
DNA which has substantially the same nucleotide sequence
as shown in SEQ ID NO:16 encoding residues 83-122.

14.   Isolated DNA according to Claim 12 comprising
nucleotide sequence SEQ ID NO:16 encoding residues 83-122.

15.   A DNA probe comprising at least 14 contiguous
nucleotides of said isolated DNA of Claim 14 or the
complement thereof.

16. Isolated DNA capable of hybridizing under high stringency conditions to said isolated DNA of Claim 14 or to the complement thereof.

17. Isolated DNA capable of hybridizing under high stringency conditions to DNA encoding a peptide of Claim 1.

18. Isolated DNA which encodes a peptide according to Claim 2.

19. A method for screening for ligands for CRF receptors, which method comprises

carrying out a competitive binding assay with a CRF receptor, a peptide according to Claim 1 which contains a suitable label, and a candidate ligand and

determining the ability of said candidate ligand to displace said labelled peptide.

20. A screening method according to Claim 19 wherein said CRF receptor is CRF Receptor 2 and said labelled peptide is ^{125}I-Tyr^o-Ucn.

21. A method of diagnosing for hypothalamic pituitary disease comprising

administering an effective dose of a peptide according to Claim 1 to a human subject and monitoring the bloodstream of said subject for an increase in ACTH level.

22. A method for stimulating secretion of ACTH and β-END-LI in mammals comprising

peripherally administering to said mammal an effective amount of a peptide according to Claim 1 or a nontoxic salt thereof and a pharmaceutically acceptable carrier therefor.

23. A method of modifying blood flow and/or blood pressure which comprises administering an effective amount of a peptide according to Claim 1 or of an antagonist peptide thereof.

24. A method according to Claim 23 for modulating blood flow in a desired vascular bed which comprises peripherally administering said effective peptide amount.

25. A method of increasing coronary blood flow which comprises peripherally administering an effective amount of a peptide according to Claim 1.

26. A method of decreasing swelling and/or inflammation and/or vascular permeability which comprises parenterally administering an effective amount of a peptide according to Claim 1.

27. A pharmaceutical composition which comprises an effective amount of a peptide according to Claim 1 in combination with a pharmaceutically acceptable carrier, which amount is effective to modulate the transactivation of CRF receptors.

28. A pharmaceutical composition according to Claim 27 wherein said amount is effective to increase intestinal transit rate.

29. A Ucn antagonist peptide which comprises the following amino acid sequence:
Asp-Leu-R_{10}-R_{11}-His-Leu-Leu-Arg-Thr-Leu-Leu-R_{19}-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-R_{29}-Ala-Glu-R_{32}-Asn-Arg-Ile-R_{36}-Phe-R_{38}-Ser-Val-NH_2, wherein R_{10} is Pro, D-Pro, Thr or D-Tyr; R_{11} is D-Phe or D-Tyr or another D-amino acid; R_{19} is Glu or Ala; R_{29} is Arg, Glu, Lys or Orn; R_{32} is Gln, Lys, Orn or

Glu; R_{36} is Ile, C^{α}MeIle or C^{α}MeLeu; R_{38} is Asp or Ala; provided that when R_{29} is Glu, R_{32} is either Lys or Orn and the side chains thereof are linked by an amide bond and that when R_{32} is Glu, R_{29} is either Lys or Orn and the side chains thereof are linked by an amide bond; and provided further that Glu in the 31-position can be D-Glu or another D-amino acid and that the N-terminus can be shortened by 1, 2 or 3 residues.

30.   A Ucn antagonist peptide according to Claim 29 which is (cyclo 29-32)D-Phe^{11},Glu^{29},Lys^{32}-Ucn(11-40).

31.   A method for screening for antagonists for CRF receptors which bind with high affinity to such receptors but do not substantially activate such receptors, which method comprises

carrying out a competitive binding assay with a CRF receptor, a peptide according to Claim 30 which contains a suitable label, and a candidate antagonist and determining the ability of said candidate antagonist to displace said labelled peptide.

32.   A peptide useful for blocking CRF-binding protein to thereby increase availability of endogenous CRF and/or Ucn, which peptide has an amino acid sequence that is substantially the same as:
Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln (residues 3-32 of SEQ ID NO:15), or a biologically active fragment thereof which is formed by deleting 1 to 8 residues in sequence from the N-terminus, or 1 to 5 residues in sequence from the C-terminus, or both.

-88-

33.   A peptide according to Claim 32 selected from the group consisting of Ucn(5-32), Ucn(8-32) and hUcn(3-27).

34.   A method for increasing the *in vivo* level of CRF and/or Ucn, which method comprises administering an effective amount of a peptide according to Claim 32.

35.   A method according to Claim 34 wherein said effective amount is sufficient to promote parturition in a pregnant female.

36.   A method according to Claim 34 wherein said amount of said peptide administered is effective so as to result in an increase in free endogenous CRF and/or Ucn in the brain which causes (a) improvement in short to medium term memory in a subject afflicted with Alzheimer's disease; (b) relief from chronic fatigue syndrome; (c) suppression of appetite; (d) stimulation of the respiratory system, (e) improvement in learning performance; (f) improvement in memory; (g) improvement in alertness; (h) reduction of depression and/or (i) lessening of anxiety.

37.   A method according to Claim 36 wherein said peptide is administered so that said peptide reaches the brain.